THE UNIVERSITY OF ALBERTA

"Germination, Extreme Temperature Resistance, and CO_2 Gas Exchange of Three Grass Species with Potential Use in Reclamation"

by

©

Heather D. Addy

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IN

Plant Ecology

Department of Botany

EDMONTON, ALBERTA

Fall 1986

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "Germination, Extreme Temperature Resistance, and CO_2 Gas Exchange of Three Grass Species with Potential Use in Reclamation" submitted by Heather D. Addy in partial fulfilment of the requirements for the degree of Master of Science in Plant Ecology.

Walter Moser
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John Hoddinott

Date 1986 - 09 - 18

Abstract

Reclamation of disturbances in alpine regions is made difficult by climatic and physical features of the environment: the cool, short growing season, high winds, steep topography, and late summer drought all impair both natural recovery of damaged areas, and man's attempts at reclamation. Special reclamation techniques are required in these areas, linking the physiological adaptations of plant species with the specific characteristics of the disturbed environment. This approach was follows in the present study which involved (1) examination of one environmental characteristic, the temperature regime, in a coal mined area in the Alberta Rocky Mountains, and (2) a study of the physiological adaptations and limitations with respect to temperature of grass species previously suggested for use in alpine revegetation. Both indigenous (*Poa alpina* and *Trisetum spicatum*) and introduced (*Poa pratensis* variety Nugget) grass species were used, based on the theory that the species most successful in alpine revegetation will not necessarily be those native to the area, but rather those that are best adapted to the post-disturbance conditions, which often are drastically different from the original conditions. In addition, three populations of *P. alpina* from an elevational gradient were examined to determine if local differentiation had occurred; such differentiation would have important implications for this species' use in reclamation.

Experiments were conducted to compare the three species on the basis of: (1) germination characteristics, *i.e.* their response to different light and temperature regimes to determine optimum germination conditions for each species; (2) ability to survive exposure to extreme low and high temperatures which occur on alpine disturbances; and (3) CO_2 gas exchange response to temperature, especially to low temperatures that dominate the alpine growing season.

P. alpina had the best performance in terms of percentage germination, rate of germination, and early seedling development, followed by *T. spicatum*. Scarification of the seeds of all three species dramatically increased percentage germination and rate of germination.

The three species were not very different with respect to frost damage, but *T. spicatum* had greater frost avoidance than did the other two species. *P. pratensis* had greater heat resistance. There was little evidence for a significant difference among the

P. alpina populations in terms of resistance to temperature extremes.

The native grasses have lower optimum temperatures for photosynthesis and are photosynthetically active over a broader temperature range than the introduced species. *P. pratensis* has higher net photosynthetic rates within its narrow favorable temperature range. Only *T.spicatum* displayed true photosynthetic acclimation to temperature over a broad temperature range. There was little evidence for differentiation among the populations of *P. alpina*

Given that the most important adaptation for survival and propagation in alpine areas is the ability to photosynthesize and grow at low temperatures, the two native species would be more successful than the introduced species. Since *T. spicatum* has the additional advantage of high acclimation potential, this species shows the greatest promise of the three species studied for use in revegetation of alpine disturbances, on the basis of these experiments.

Acknowledgements

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1. General Introduction

1.1 Alpine Regions

Two of the most common terms used to describe alpine environments are "severe" and "fragile". Certainly for most animal and plant species, alpine conditions are severe.In Banff, located in the Eastern slopes of the Canadian Rocky Mountains (Fig. I-1), the average yearly temperature is less than 10° C, and several months of the year have mean temperatures below 0° C (Alberta Environment Report for 1983; for an explanation of climate-diagrams, see the Appendix.) At higher elevations, therefore, the growing season is very short, frequently less than 90 days (Billings 1973, Luttmerding 1976). Drought is common in the alpine during the latter part of the growing season (Mayo *et al*. 1977) and wind speeds are very high, usually twice those of the forested subalpine zone (Ogilvie 1969). These features, combined with the cool climate, greatly restrict plant productivity, such that most plant species cannot survive and propagate in the alpine.

There is however, a small number of plant species which are adapted to the cool temperature regime, and for these species, the environment is not severe (Billings 1974a and 1974b). These plants are often unable to grow in warmer, moister areas as they are outcompeted by other species and tend to have abnormal metabolism at temperatures above 25°C (Billings 1974a). Dominant among these plants are perennial grasses and herbaceous species (Fig. I-2). Annuals are least common, as their need to complete their entire life cycle within one growing season places them at a disadvantage in alpine environments. (Billings 1973).

Although alpine plants belong to several different plant families, they share many common adaptations to their environment. Among these adaptations are: (1) a prostrate growth habit, so that the plant is close to the ground to minimize exposure to high winds and cold temperatures; (2) extensive root and rhizome systems, for underground storage of compounds such as carbohydrates and oils. These compounds enable the plants to grow rapidly at the start of the next growing season. (3) Preformed shoot and flower buds that allow for rapid shoot elongation and flowering to occur very soon after temperatures increase in the spring; and (4) efficient metabolism at low

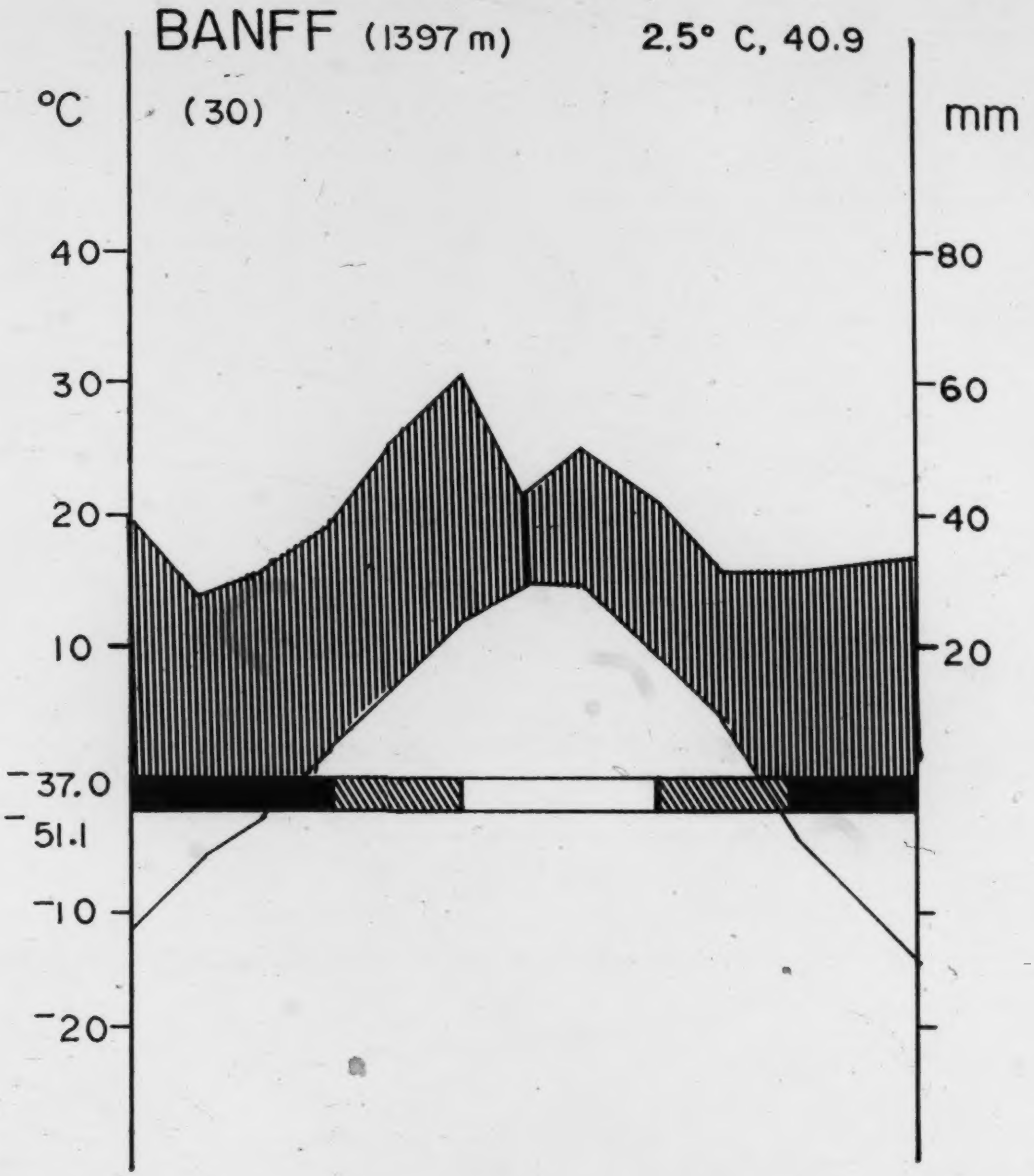


Fig. 1-1. Climate-diagram for Banff, located in the Eastern slopes region of the Alberta Rocky Mountains. See text for explanation. Data from Alberta Environment Report for 1983.

Page 3 has been removed because of the unavailability of copyright permission. Page 3 contained a listing of alpine life forms, in order of importance, from Billings (1978).

temperatures , so that these plants can complete their life cycle in the cool alpine temperature regime (Billings 1974a and 1974b). Although most alpine species have this high metabolic rate at low temperatures, they are limited by the brief duration of favorable conditions for growth, and so tend to be slow-growing. Therefore, natural succession and colonization tends to be a slow process in the alpine. Natural recovery of a disturbed alpine area takes a very long time, if indeed it ever occurs (Billings 1973, Roach and Marchand 1984). It is for this reason that the alpine region is considered fragile.

1.2 Disturbances and Reclamation

Although alpine regions occupy less than ten percent of the province of Alberta (Strong and Leggat 1981), their geographical size is in no way proportional either to their importance or to the impact that disturbances have on them. Mountains provide man with timber, water, hydroelectric power, and minerals; and they are an essential wildlife habitat. These areas are also important for aesthetic reasons: they are an area for a multitude of recreational activities, and they possess incredible beauty and splendour.

Increasingly, alpine areas are the scene of recreational and industrial disturbances, which result in damage to the soil and vegetation of alpine habitats. In Alberta, one of the major sources of disruption is the mining industry; of the total area covered by the Rocky Mountains in this province, recoverable coal seams underlie about nine percent (Sims *et al.* 1984). At present, strip mining is the predominant method used to recover this coal. Strip mining is a type of surface mining in which the overburden (i.e. the soil, rock, and other strata overlying the coal seam) is removed in cuts, one narrow band at a time (Sims *et al.* 1984). This overburden is then deposited in spoil heaps around the pit.

Provincial legislation requires mining companies to reclaim any land they have disturbed:

> "Land reclamation will include the contouring of the mined or disturbed lands, the replacement of the top soil, revegetation for soil stabilization, biological productivity and appearance, and suitable maintenance of the vegetation...
> (Alta. Dept. of Energy and Natural Resources, 1976)

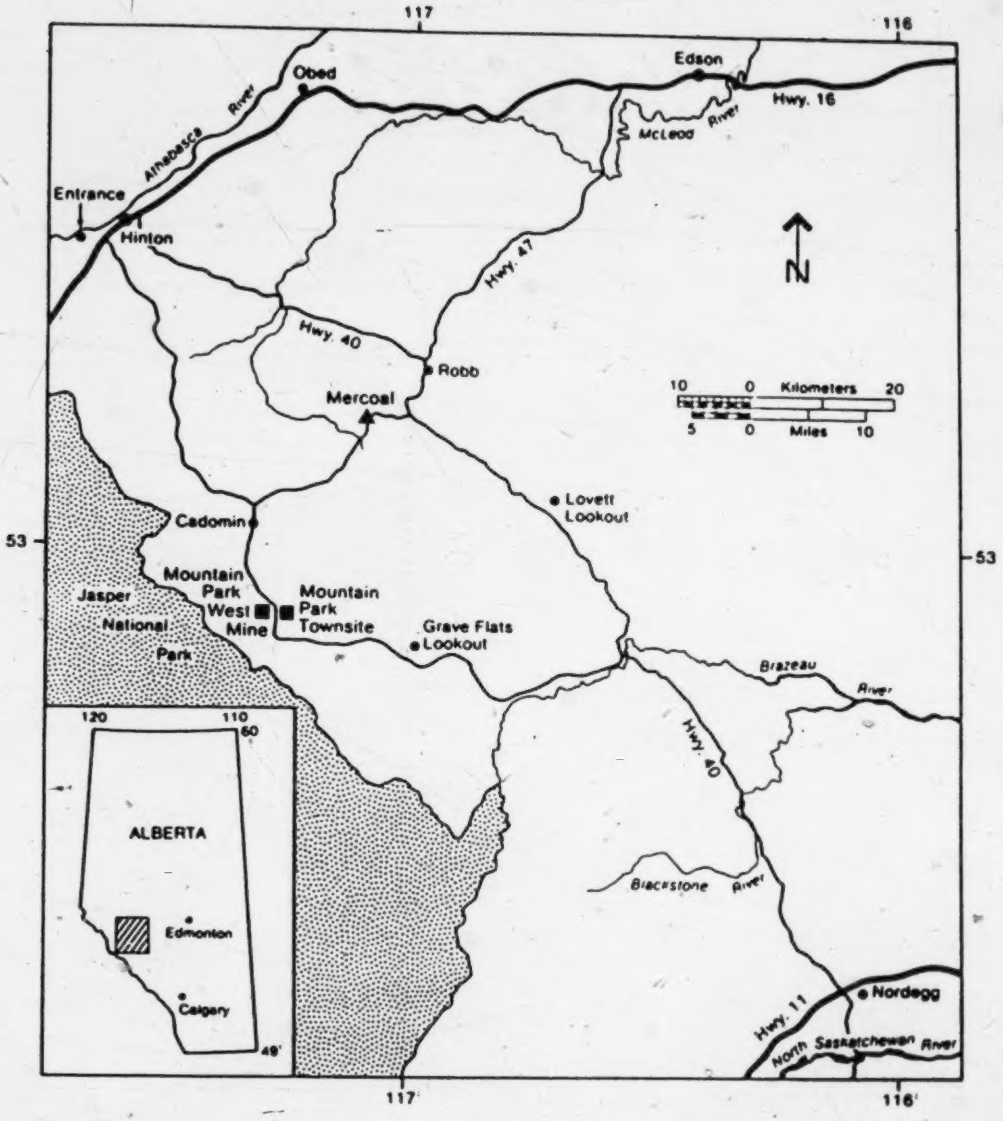
Reclamation of alpine areas is difficult due to the aspects of climate and topography discussed above; these factors will impair man's attempts at alpine revegetation just as they inhibit the natural rate of recovery. Therefore, special reclamation techniques are required in the alpine. A promising approach is that suggested by Brown and Johnston (1979):

> "The challenge in developing rehabilitation techniques for alpine disturbances is to carefully link three paramount variables: (1) overall climatic factors; (2) factors of the disturbed environment; (3) physiological adaptations of the plants."

The major goal of my research was to investigate part (3), as there is little known about the physiological requirements and limitations of alpine species. Before examining plant adaptations, it was necessary to collect information about the conditions of a disturbed alpine area, to determine the conditions that a plant must deal with in such an area. In order to obtain information about the plant environment that exists in areas to be reclaimed, a few factors critical for plant life were assessed. This quantitative assessment was the subject of this project's field work.

1.3 Field Study

The site chosen for the field study was the abandoned Mountain Park West Mine, located 26 kilometers southwest of Cadomin, Alberta (Fig. I-3). The mining operation at the Townsite Mine (elev. 1780 m) was one of the earliest underground mines in the Coal Branch of the Eastern Slopes region: it opened in 1912 and was shut down in 1949 (Ross 1974). In contrast, the West Mine (elev. 1810 m) was a strip mine in operation only from 1945 to 1950 (Ross 1974). Neither site was revegetated or reclaimed (Lake 1967). At the West Mine, two pits had been excavated and the overburden (rock, soil, and mining wastes) was dumped over a 34 ha area to the north in a series of spoil heaps. The heaps are more or less level on top, with long, steeply sloping sides. Research done by Russell (1980) shows that the spoil heaps consist of virtually coalfree overburden, and have been only sparsely colonized. The dominant plants on the West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5 0 Miles 10
Lovett Lookout
Cadomin
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Mountain Park West Mine
Mountain Park Townsite
Jasper National Park
Grave Flats Lookout
Brazeau River
120
110
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ALBERTA
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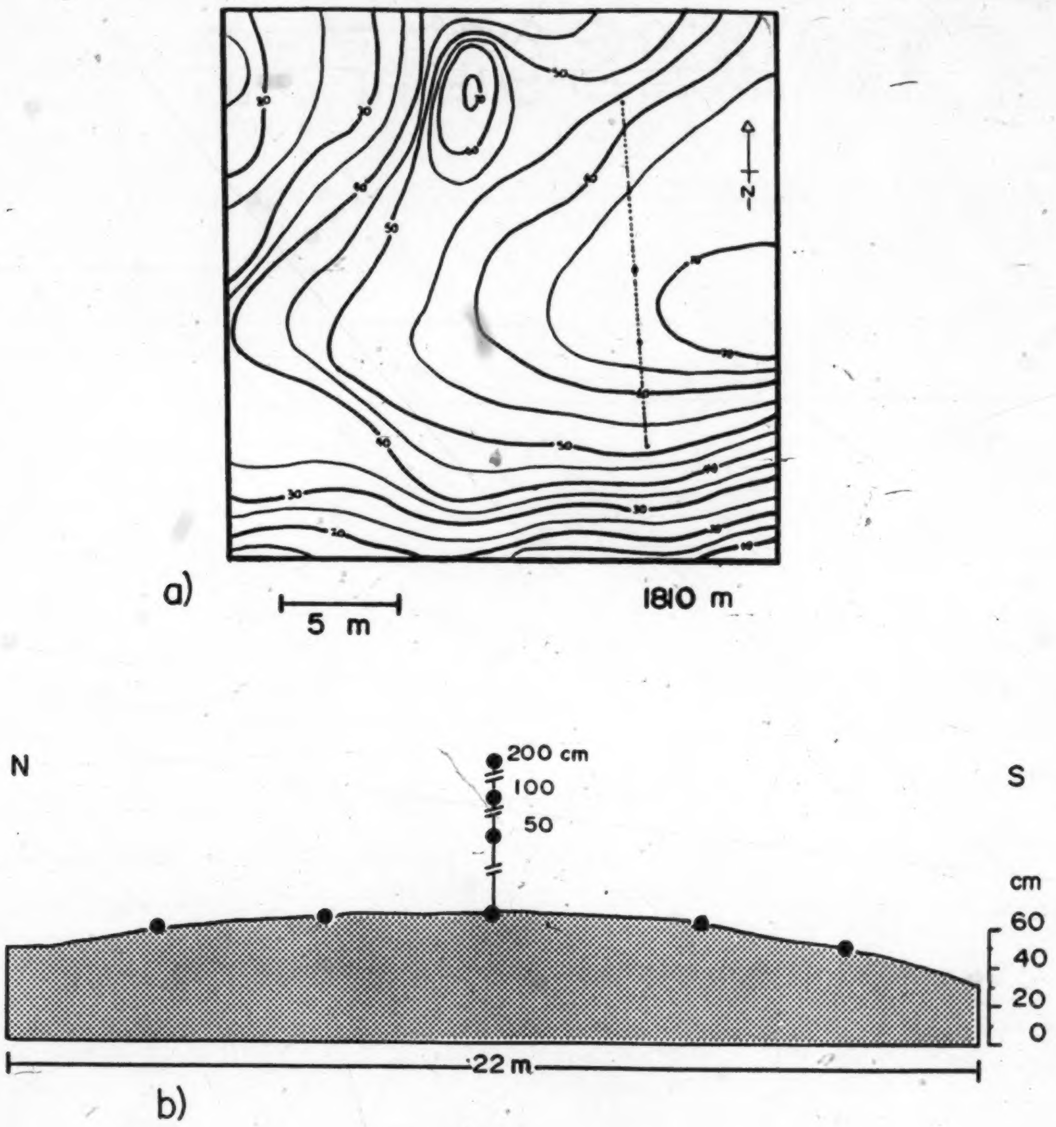
Calgary
49
Hwy. 40
Blackstone River
Nordegg
Hwy. 11
North Saskatchewan River
117
116

Fig. 1-3. Location of the field study area in the Eastern Slopes region of the Alberta Rocky Mountains. Adapted from Russell 1980.

Mine spoils are tuft-forming grasses such as *Agropyron latiglume* (Scribn. & Smith), *Poa alpina* (L.), *Trisetum spicatum* (L.)Richt., and herbaceous dicots such as *Trifolium repens* (L.) (Russell 1980).

Review of the literature indicated that the temperature regime is one of the critical factors affecting plant establishment on open disturbed sites such as these spoils (Bramble and Ashley 1955, Schramm 1966). Therefore, temperature relations of air, leaves, spoil surface and subsurface layers were monitored. A site with a moderate amount of established vegetation was selected to monitor these temperature relations and a 15 m transect running from northwest to southeast was established (Fig. I-4a). Air temperature was measured at three heights (.5 m, 1 m, 2 m) in the center of the transect, and spoil surface temperature was measured at five locations along the transect (Fig. I-4b). These measurements were taken continuously using a Campbell Scientific CR-21 Programmable Micrologger with thermistors. Leaf and subsurface temperatures were measured at three locations along the transect using copper-constantan thermocouples and a microvolt meter; readings were taken every 15 minutes from early morning to late afternoon. The thermocouples were placed into the spoil using a modification of the method suggested by Fritschen and Gay (1980) (Fig. I-5); in this method, a shallow hole was dug adjacent to the measurement site, and the thermocouples were inserted horizontally into the soil, to minimize disturbance of soil layers. The soil was then replaced into the hole. The field work was done in July and August of 1984.

The results indicate the extreme temperature variations possible during the growing season on such open disturbed sites. July of 1984 was a warm month: daytime air temperatures often reached 25° C (Fig. I-6). Spoil surface and leaf temperatures were very high, commonly 40° C. Subsurface temperatures were of the same magnitude, and there was no appreciable drop in temperature until ten centimeters below the surface; even at these depths, temperatures of 30° C occurred. Such


a)
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b)
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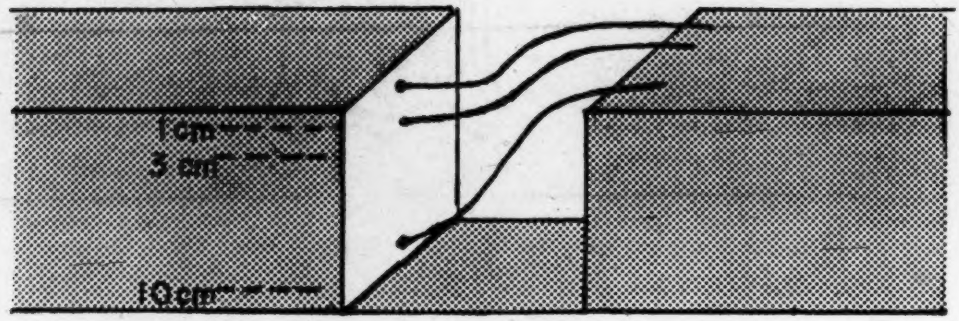
Fig. 1-4. Mountain Park West Mine study site (a) contour map, showing transect; (b) side view of sampling transect.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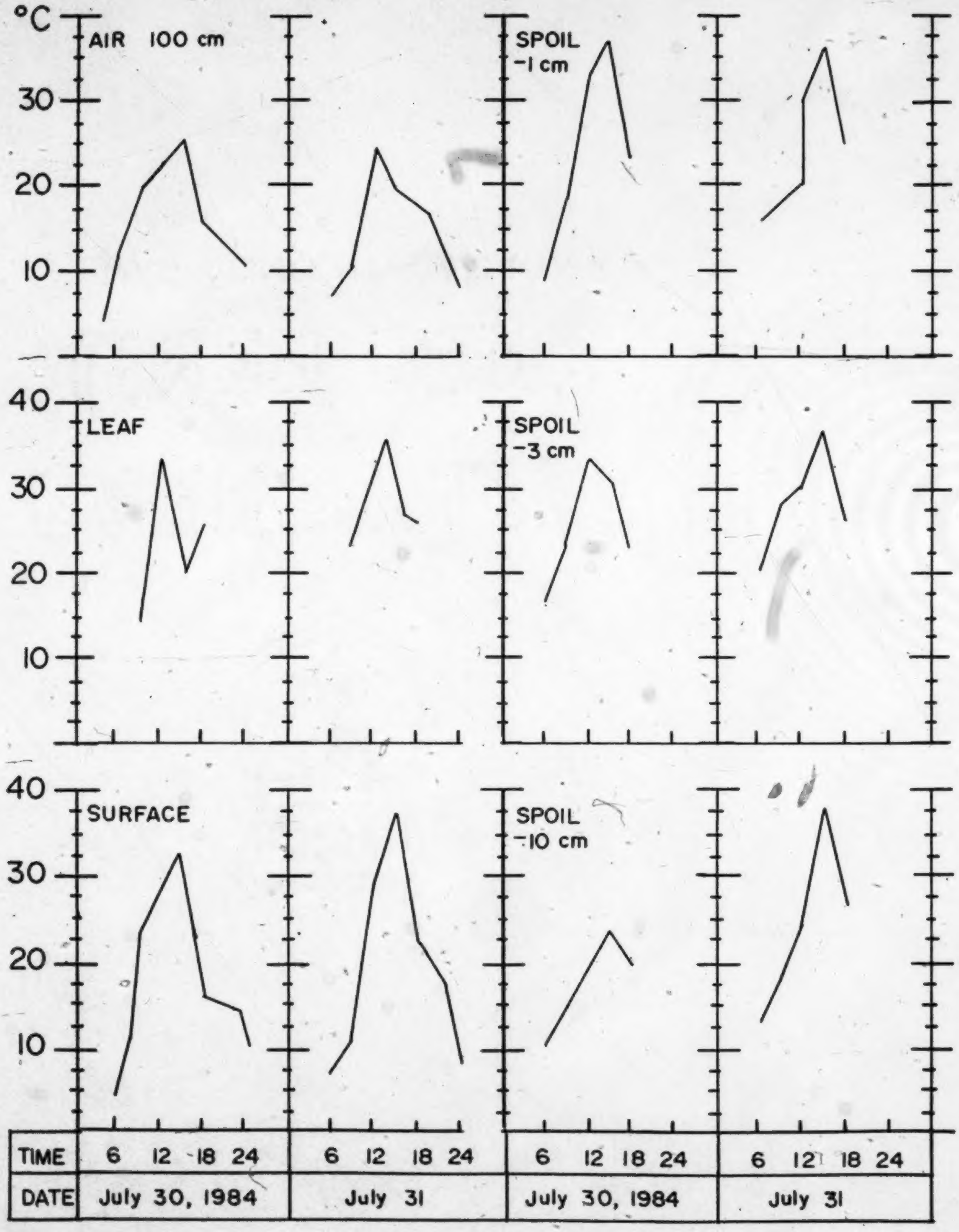
3 cm
10cm

Fig. 1-5. Sketch of soil thermocouple installation at 1, 3, and 10 cm depths.
Adapted from Fritschen and Gay 1980.

Fig. 1-6. Examples of leaf, air, and spoil temperatures at Mountain Park West Mine study site during July, 1984. Leaf and spoil temperatures are the mean of five sampling sites (see Fig. 1-4 of sampling along transect).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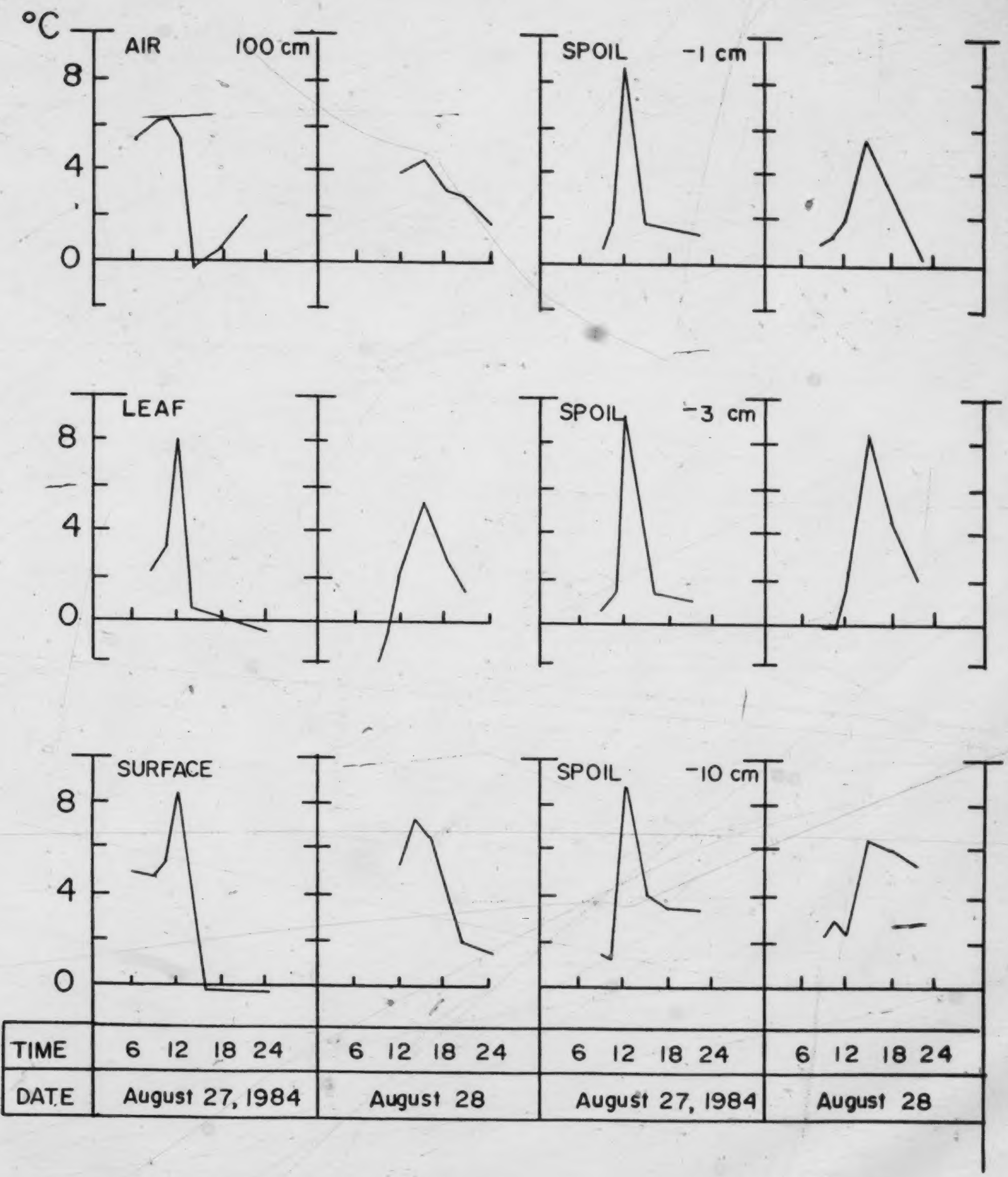
extreme surface temperatures have been found in previous studies of mine site microclimate: Grossnickle and Reid (1984) reported soil temperatures reaching 45° - 75° C on a high-elevation strip mine and Thompson and Hutnick (1972) found spoil temperatures often exceeding 50° C. These temperatures develop as a result of : (1) high solar radiation, and (2) the coarse nature of the spoil material, which thus has a negligible capillary rise of water, resulting in rapid surface drying and a steep temperature increase (Schramm 1966). Thus it is common for mine spoil temperatures to be at or near 50° C, which is considered the lethal temperature for most plant life. Schramm (1966) concluded that injury from these extreme temperatures is usually restricted to the plant parts in direct contact with the surface layers in which radiation is converted to heat, and thus is where the highest temperatures occur. The majority of roots, which are very sensitive to heat damage (Larcher 1980), are below this zone and so usually are not exposed to lethal temperatures. However, on the Mountain Park spoils, the surface layers around the base of grass tufts are eroded by wind, exposing the roots to these extreme surface temperatures. Therefore, high surface temperatures could be a limiting factor in plant establishment and survival on these spoil heaps.

August of 1984 was a cooler month, and on the 28 of August, the study area recieved 25 centimeters of wet snow. This snow persisted for the next five days, resulting in air and soil temperatures below zero (Fig. I-7). These data indicate that plants face the possibility of freezing temperatures during the growing season on high-elevation open sites.

1.4 Selection of Plant Species

There is much debate at present about the relative merits of native versus introduced species in reclamation. Agronomic species that have been successful in reclaiming lowland disturbances often do not do well at high elevations (Ziemkiewicz 1982), largely because they have been bred for characters that maximize productivity

Fig. 1-7. Examples of leaf, air, and spoil temperatures at Mountain Park West Mine study site during August, 1984. Leaf and spoil temperatures are the mean of five sampling sites (see Fig. 1-4 of sampling transect).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but are not advantageous in the alpine environment (Walker 1979). For example, agronomics have the majority of their biomass above ground (Ziemkiewicz 1982) and have low shattering at harvest time (Walker 1979); neither of these traits would be advantageous in alpine regions. The carbon balance of a native species is oriented towards reproduction ensuring genetic continuity in an environment, whereas in introduced species, which are often agronomic plants, man is attempting to maximize the harvestable yield. This has resulted in different patterns of photosynthesis, assimilate partitioning and dry matter production (Saugier 1983). Therefore, introduced agronomic species, if used for reclamation, are being used for a purpose to which they are not well adapated.

Thus it has often been advocated that native grasses be used to reclaim high elevation disturbed lands (Aldon and Springfield 1975, Cook 1976, Etter 1973, Younkin 1972). These plants already possess the adaptations required for survival in the alpine and tend to have characters important in reclamation, such as longevity and tolerance of low soil fertility (Cuany 1974). Native species certainly represent a good starting point for selection of reclamation species; however, not all species that originally grew on a site will be equally suited for colonization after disturbance, as environmental conditions are often drastically altered, e.g. soil water-holding capacity greatly decreases (Bunza 1978; Körner *et al.* 1978). Therefore, the key consideration in species selection should not be the area of origin of the species, but rather its suitability to the present altered environment. Thus, I chose to work with three species: two native alpine grasses, *Poa alpina* L. and *Trisetum spicatum* (L.) Richt., and one introduced grass species, *Poa pratensis* L.

P. alpina, a perennial tuftforming grass, has a circumpolar distribution and is important in arctic and alpine regions of North America (Moss 1983). It is common on both dry and moist slopes in the mountains, and tolerates extreme environments such as bare soils and talus slopes (Hitchcock and Cronquist 1973, Moss 1983). *T. spicatum*, like *P. alpina,* is a perennial tufted bunchgrass, and is found in the arctic ranging from Alaska eastward to Hudson Bay, and from the Rocky Mountains south to California (Moss 1983). In alpine regions it is common in dry places such as rocky outcrops, but is also found in meadows (Hitchcock and Cronquist 1973, Moss 1983). Both of these native grasses commonly invade disturbed alpine and subalpine areas (Walker 1979). The third grass species, *Poa pratensis* , was introduced to North America from Europe before 1700 and rapidly spread westward, becoming so common that it is often considered to be a native (Elliott and Bolton 1970). *P. pratensis* is a sod-forming, rhizomatous perennial, and is especially well adapted to a cool, humid climate , growing on well-drained soils in meadows or open woods (Hanson 1972, Hitchcock and Cronquist 1973). The particular variety used in this research, *P. pratensis* variety Nugget, originated from breeding stock near Hope, Alaska (Elliott and Bolton 1970). It has been extensively used for pasture, recreational turf and erosion control in temperate areas, and has been recommended for use in reclamation after its success in low-elevation revegetation (Hanson 1972).

It is often assumed that native plants are so intimately adapted to their local environment that if transported to another area, they will not do as well. If this were true for these native grasses, they would be less suitable for reclamation as a separate specialized population would be required for each local area (Walker 1979). While such elaborate pairing of plants with habitats may be of some benefit in theory, since each area could be revegetated with the population best adapted to that area (Billings 1973), it would make reclamation much more costly and complicated (Walker 1979). For this reason, it was decided to examine one of the widely distributed native species, *Poa alpina*, to determine if such specialized local populations have developed.

1.4.1 Agamotypic Differentiation

Turesson's pioneer work (1922) on the interaction of genotype and environment initiated a new area of botanical research and a new methodology for studying variation within a species. He noted that populations of a species occupying different areas often displayed distinctive morphological and physiological traits, which frequently were adaptive. In several studies (1922), Turesson collected samples of populations from different areas of a species' range, and grew them under uniform conditions. Over the next several years, he observed certain morphological characteristics of the different populations and observed how most traits persisted, and thus were likely due to genetic differentiation between the populations. Moreover, Turesson demonstrated that these differences were correlated with the original habitat of the population. For such genetic races, he proposed the term "ecotype", defined as "...the product arising as a result of the genotypical response of a species to its particular habitat". Since Turesson, numerous researchers have carried out similar transplanting experiments and have shown that many wide-ranging species display such ecotypic differentiation (Clausen, Keck, and Hiesey 1940, Mooney and Billings 1961, Pearcy *et al* 1977). Turesson later (1943) turned his attention to the variation evident in populations of apomictic species. "Apomixis" is a collective term for all types of asexual reproduction that supplement or replace sexual reproduction (Valentine 1960). This includes reproduction by vegetative means such as stolons,and runners, as well as vivipary and agamospermy (embryo formation without fertilization). It is this last type that is of greatest concern in the present research.

Agamospermy occurs in three major ways: by diplospory, apospory, or pseudogamy. The process of diplospory involves an embryo sac mother cell which enlarges and divides mitotically, resulting in a normal but unreduced embryo sac. In apospory, while the embryo sac mother cell undergoes meiosis, a nucellar cell divides mitotically; this unreduced embryo sac outcompetes the reduced one, and forms an embryo. Pseudogamous species require fertilization for endosperm development, but the embryo develops parthenogenetically (Grun 1955). Thus it would seem unlikely for an apomictic species to possess locally differentiated populations, if one uses the strict genetical meaning of population as there is no gene exchange possible between biotypes or between members of the same biotype (a biotype is an assemblage of individuals with essentially the same genotype within populations) (Davis and Heywood 1963). However if "population" is defined in a broader taxonomic sense, as Davis and Heywood suggest, meaning morphologically similar groups of plants of the same species growing in a certain area, then apomictic populations are found to consist of more than one biotype. Davis and Heywood point out that while morphological differences may not be very apparent, the physiologies may be very different. How does such variation arise in apomictic species?

Apomixis may be facultative or obligate. In facultative plants both reduced and unreduced embryo sacs occur and thus both apomictic and sexual offspring are possible. The embryo sacs of obligate plants are all unreduced, meaning no sexual offspring are possible, but new variants arise by means of mutation, autosegregation, and other chromosomal changes (Valentine 1960). Furthermore, these obligate apomicts without any sexual reproduction are much rarer than previously believed (Grun 1955, Marklund and Rousi 1961). Thus, new biotypes can occur in apomictic species, and some of these may separate into different ecological niches and become specialized to their local environments. Turesson (1943) concluded that the biotypes of apomictic *Alchemilla* microspecies show much the same behaviour as the biotypes of sexual species, and that their extensive distribution was primarily due to the fact that they are composed of a number of genetically distinct types. Turesson termed these "agamotypes", defined as climatically or edaphically specialized biotypes equivalent to "ecotypes" in a sexual species (Turesson 1943). The term "agamotype" is not very commonly used; usually, the term "ecotype" is used for both sexual and apomictic species.

Turesson later performed progeny tests on the *Alchemilla* microspecies and found that the morphological and physiological differences between the agamotypes were retained in their progeny (Turesson 1956). Bradshaw (1963) also studied *Alchemilla* species and his results agreed with those of Turesson: the characteristics of the parents were preserved in their offspring, and variation was related to environmental factors. Thus apomictic species, long regarded as uniform and monotypic certainly can be heterogenous.

Poa alpina is an apomictic species that has been extensively studied in both Europe and North America. In this species, the degree of agamospermy is rarely or never absolute (Muntzing 1966). Most plants of *P. alpina* are facultative apomicts, and tend to be diplosporous or pseudogamous (Löve 1960), although pseudovivipary (a type of asexual reproduction in which propagules replace flowers in the inflorescence) is common (Davis and Heywood 1963). Grun (1955) did a cytogenetic study of several *Poa* species and found that in the facultative apomict *P. alpina*, several previous studies showed some individual plants to be strongly or entirely sexual. Even in obligate apomictic species such as *P. nervosa*, plants from geographically isolated localities were distinct both cytologically and morphologically. While Grun did not examine any physiological characters, adaptations are much more likely to be expressed in physiological rather than morphological differences shown by an individual or population (Davis and Heywood 1963). *P. alpina* populations collected from several areas in middle Sweden by Muntzing (1966) displayed a marked variation in chromosome number, ranging from the diploid number 14 to about 50. Muntzing (1966) concluded that "...an explosive mass production of new biotypes has been shown to occur in an area which is otherwise dominated by apomixis". Although the majority of Scandinavian *P. alpina* populations are largely apomictic, there is still a sufficient degree of sexuality left to maintain and enrich the polymorphism of the species.

1.5 Collection of *Poa alpina* populations

The *Poa alpina* populations were collected by the Vegetation Group working in land reclamation research and development at the Alberta Environmental Centre at Vegreville, Alberta. The plants were collected from 2700 metres, 2300 metres, and 2000 metres ASL on Fairview Mountain, Lake Louise in 1984. All collection sites were in the Alpine and Subalpine ecoregions of Alberta (Strong and Leggat, 1981). The highest elevation site is located in an alpine fell-field, with coarse well-drained soils and conditions ranging from cool/wet to warm/dry. The vegetation is an intermixture of *Cassiope tetragona* and *Carex spp.* in the wetter sites, and *Dryas hookeriana* with *Phyllodoce empetriformis* in the drier areas. *Festuca saximontana* and *Trisetum spicatum* were also found at this site. The collection site at 2300 m is located on a level saddle area, also with coarse well-drained soil. The dominant plant species at this site were *Kobresia spp., Koeleria cristata, Festuca saximontana*, and *Trisetum spicatum*. The lowest site is located in a valley bottom in the Spruce-Fir subregion, dominated by mixed stands of *Abies lasiocarpa* and *Picea engelmannii*. *Poa alpina* is found here only in disturbed locations such as along trailsides. (Above information on sites provided by R. Hermesh, Head of the Vegetation Group).

1.5.1 Handling of the plants

Ten plants of each population were divided between a "warm regime" growth chamber and a "cool regime" growth chamber. In the warm regime, plants received a 16 hour photoperiod, combined fluorescent-incandescent illumination and a thermoperiod of 25° C days and 5° C nights. Under the cold regime conditions, the plants received the same photoperiod, illumination, and night temperature, but the daytime temperature was 10° C. Relative humidity, while not controlled, remained constant at 60%. All pots were well watered so that the soil water potential was assumed to be near zero at all times. Plants were given full strength Hoagland's solution twice a week.

1.6 Hypotheses

Overall, the data obtained from the field study reveal the importance of the temperature regime as a factor limiting plant colonization of alpine disturbances such as mine sites. Following the suggestion of Brown and Johnston (1979), the major physiological adaptations essential for plant establishment, survival, and propagation under these conditions were investigated. The background information collected on Mountain Park West Mine indicates that grass species which would be successful in reclamation of such alpine disturbances should possess a few vital characteristics; i.e. they should (1) be able to germinate at the low temperatures which prevail at the start of the growing season in alpine areas; (2) be able to survive low and high temperature extremes; and (3) have a capacity for photosynthesis over a wide temperature range and high photosynthetic activity at low temperatures. The examination of each hypothesis will be dealt with in separate chapters of the thesis.

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2. Germination and Establishment

2.1 Introduction

Several native plant species have been studied to identify and elucidate adaptive physiological mechanisms that enable these plants to survive in the alpine region. Summaries of such studies have been made by Bliss (1962), Johnson (1969), and Billings (1973); these summaries have characterized the alpine environment in terms of factors that may be limiting to plant survival, growth, and reproduction. The most important factors are: low temperature, high wind, short and variable growing season, variable snow depth, and often, deficient mineral nutrient supplies. These factors provide the selective pressures that have resulted in the observed plant adaptations (Clebsch and Billings 1976).

The processes of growth and establishment are of critical importance in the life cycle of alpine plants; as Thienemann's Rule states, the most sensitive stages of the plant life cycle , such as the young seedling, set the limits for the maintenance and spread of a species (Larcher 1980). This study deals with lab studies of seed germination in relation to temperature and light factors in two indigenous alpine, and one introduced grass species.

Germination is influenced by several internal and external factors; among these are temperature, water availability and light, which trigger a series of morphological changes that convert a seed to a seedling. This transformation can be summarized in five stages: (1) imbibition, the adsorption of water onto the internal surfaces of the seed, causing swelling of colloidal materials; (2) hydration and biochemical activation; (3) cell division and elongation; (4) emergence of the radicle from the seed; and (5) establishment of the plant primary body (Berlyn 1972).

2.1.1 Viability

Seed germination and viability in alpine species has been the subject of several studies. Pelton (1956) examined 18 alpine species and found that all set viable seed; Bliss (1959) studied 26 species of which 96% produced viable seed. Of the six alpine species examined by Sayers and Ward (1966), four had greater than 90% viability. There is evidence for intraspecific variation of viability; for example, Amen (1966) found a decrease in viability with increasing elevation.

2.1.2 Dormancy

Seeds are considered to be dormant when they do not germinate under conditions normally regarded as favorable to germination, i.e. when there is a plentiful water supply, suitable temperature and normal atmospheric composition (Mayer and Poljakoff-Mayber 1963). Dormancy can be due to a variety of causes: immaturity of the embryo, an impermeable seed coat, special light or temperature requirements, or the presence of inhibitory chemicals. Given the physical conditions of the alpine environment discussed above, it might be expected that there would be a common low-temperature requirement for the germination of alpine seeds. However, experiments have shown that dormancy is quite rare and that very few of these dormant species require a cold-treatment. Of the approximately 62 species tested by Pelton (1956), Amen (1966), Bliss (1959), and Bonde (1965), about 60% exhibited germinability upon ripening and only 40% appeared to be actually dormant; most of the dormant species were sedges,and willows. Of those that germinated, 19 species had greater than 50% germinability (Amen, 1966). The lack of a cold requirement might be due to the insulating effects of the snow cover, or as Amen (1966) suggests, a temperature-controlled dormancy may not be advantageous for seed survival in areas with such brief growing seasons. The most common cause of dormancy among alpine species seems to be seed coat inhibition (Bliss, 1971), as several workers have reported that scarification promotes germination

(Pelton 1956, Amen and Bonde 1964). Seed dormancy thus seems to be controlled by the tundra environment, especially low winter temperatures. Seeds usually would mature too late in the season that they are produced to meet the correct requirements of temperature and moisture for germination; the exception to this situation would be the seeds of wet-site plants, such as willows and sedges, which would have abundant moisture even late in the season; this may be the reason why these seeds exhibited dormancy.

2.1.3 Early seedling growth

For survival in both the alpine and disturbed sites, it is essential for a seedling to produce a large, well-developed root system. The underground parts of most alpine perennials have two to six times greater dry weights than stems and leaves, and some (*e.g. Ranunculus glacialis*), up to ten times greater root mass (Moser *et al* 1977), and thus can act as carbohydrate storage organs (Mooney and Billings 1961, Scott and Billings 1964). Secondly, early root development is related to successful establishment in several graminoid species. Plummer (1943) studied the germination and development of seedlings of 12 grasses, and found that root development prior to summer drought appears to be directly related to initial success or failure of the seedlings. Species that were known to be difficult to establish were found to be those with slow root development. The establishment of a good root system is an important criterion for species suitable for reclamation. Root growth into spoil materials contributes to the stabilization of disturbed sites, enhances soil development and improves the chances for plant survival during droughts (Nicholas and McGinnies 1982).

2.1.4 Study objectives

The first objective of this study was to determine the temperature response of germination and early seedling development, and the interaction between temperature and light in three graminoid species: *Poa alpina, Trisetum spicatum,* and *Poa pratensis* variety Nugget. Given the short cool growing season of the alpine, and the lack of a requirement for stratification in most alpine species, it was hypothesized that the seeds would germinate early in the growing season, as soon as there was a slight temperature increase. This adaptation would be valuable in the alpine, as it is important for the plant to become established early in the season, so that it will have time to mature before the return of temperatures constantly below zero. Thus a secondary objective of this study is to compare the germination strategies of these species, to provide the basis for a direct-seeding strategy useful in reclamation. The final objective was to compare the early seedling development of the three species with respect to rate of extension of root and shoot, and root:shoot biomass ratios. These three factors are useful criteria in determining seedling vigor (Whalley *et al.* 1966).

2.2 Materials and Methods

Seeds of *Poa pratensis* variety Nugget were provided by Prarie Seeds Company; *Trisetum spicatum* seeds, by Alberta Environmental Center in Vegreville. *Poa alpina* seeds were provided by David Walker of Walker and Associates, Ltd; these were collected at Coal Valley, Alberta. All seeds were stored dry at 10° C. Germination tests were carried out in petri dishes, lined with two pieces of filter paper, to which ten millilitres of distilled water was initially added. Water was added as necessary to keep the paper moist. The conditions under which germination was studied were as follows: (1) continuous light over a temperature range from 10° C to 30° C; (2) continuous darkness over the same temperature range; (3) continuous light and continuous darkness at diurnally fluctuating temperatures of 10°day/5° night ("cool" regime) or 25° day/5° night ("warm" regime). It was found that the floral bracts of both *Poa* species were easily removed by gentle friction, i.e. by rubbing the seeds between fingers. It was then decided to test the effect of the removal of the floral bracts on germination. An efficient way of removing these bracts was by rubbing the caryopses gently on very fine sandpaper. This method probably caused slight scarification of the seeds. Originally, *T. spicatum* seeds were not given this treatment, as a previous study found no effect of floral bract remoal on the germination of this species (Clebsch and Billings 1976). However, in the present experiments, the percentage germination of untreated *T. spicatum* was low, and so in later experiments the bracts were removed from this species as well.

Germination was considered to have occurred upon emergence of the radicle. Originally, each experiment ran for two weeks and the results indicate that the majority of the seeds germinated within this time. However, since the International Rules on Seed Testing (Anon. 1976) recommend that seeds for *P. pratensis* be left for up to four weeks; an extra series of experiments was done, with the longer duration. Throughout the course of the experiment, the number of germinated seeds were counted. At the end of each two week experiment, the final number of germinated seeds was counted and converted to a percentage. Measurements were made of shoot and radicle length.

2.3 Results

Results of the germination tests under constant temperature conditions are shown in Figure II-1 and Table II-1, as are the results of scarification; the effects of alternating day/night temperatures are given in Table II-1 only. These values represent germination after 14 days in the treatment indicated. It may be that this period was too short for complete germination to have occurred; but percentage germination did not substantially increase when the experimental period was lengthened to four weeks (Table II-2). Comparison of scarified and unscarified seeds suggests that time was not a limiting factor; rather, if the seed coat was abraded, then percentage germination increased rapidly. Reasonably good germination was obtained for all species, except *Trisetum spicatum*, under one or more conditions. Maximum values ranged from 62% for *T.*

Fig. II.1. Percentage germination of both unscarified and unscarified seeds under various light and temperature conditions.

☐ 24h light

▨ 24h light scarified

■ 24h dark

■ 24h dark scarified

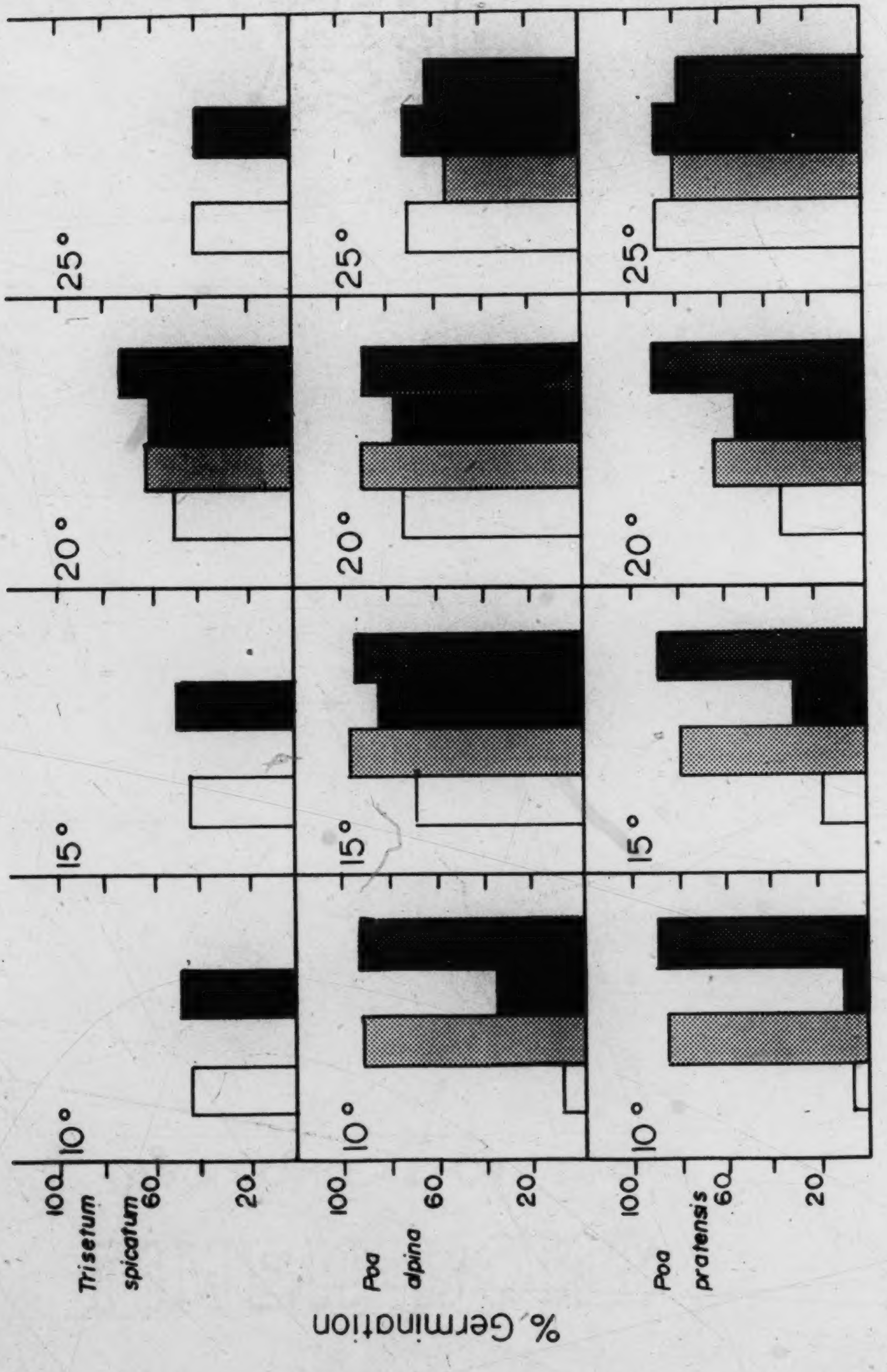
% Germination
Trisetum spicatum
Poa alpina
Poa pratensis
100
60
20
10°
15°
20°
25°

Table II-1. Percentage germination* under various light and temperature conditions.

Species	Photoperiod	24 hours				0 hours				16 hours	
	Temperature	10°	15°	20°	25°	10°	15°	20°	25°	10/5	25/5
Poa alpina	% germination	9.0	71	73.8	72	37	86	77.5	74	0.0	54
	(n=)	(75)	(50)	(45)	(25)	(75)	(50)	(25)	(25)	(25)	(25)
P. alpina (scarified)	% germination	93.3	98	92.8	56	94	96.5	92.5	64	72	96
	(n=)	(75)	(50)	(45)	(25)	(75)	(45)	(25)	(25)	(25)	(25)
Poa pratensis	% germination	6.7	20	36	87.5	10	32	56	87.5	0.0	14
	(n=)	(75)	(50)	(25)	(20)	(75)	(50)	(25)	(25)	(25)	(25)
P. pratensis (scarified)	% germination	82.6	80	63.5	80	88.7	88	90	77.5	0.0	96
	(n=)	(75)	(50)	(50)	(25)	(75)	(50)	(25)	(25)	(25)	(25)
Trisetum spicatum	% germination	46	46	63.8	44	50	52	75	42	0.0	36
	(n=)	(50)	(25)	(50)	(25)	(50)	(25)	(25)	(25)	(25)	(25)

* Values are means

Table II-2. Effect of duration of germination tests on % germination (at 20°C, 24 h photoperiod).

Species	% germination after 14 days	% germination after 28 days
Poa pratensis	32	40
P. pratensis (hulled)	50	50
Trisetum spicatum	52	56

spicatum to 98% for scarified *Poa alpina* seeds. No germination was observed at constant 5° C after 14 days; when these seeds were moved to warmer temperatures germination occurred. Results of tests at 30° C were not included, since the growth chamber temperature fluctuated greatly when the unit was set at this temperature, and the results are therefore not reliable.

Several important points are illustrated in Fig. II-1: (1) The optimum temperature for germination in *Trisetum spicatum* was 20° C, and there was considerable reduction in germination when the temperature was increased to 30° C. Once the optimum temperature range was found, the effects of lightly scarifying the seeds were tested; this treatment resulted in substantial increase in percentage germination. Germination in *T. spicatum* is also stimulated by darkness.

(2) The optimum temperature for untreated *Poa alpina* seeds was 15° - 20° C. Scarification of these seeds dramatically increased percentage germination, and broadened the optimum temperature range for germination such that 95-100% of the seeds germinated at 10° C compared with less than 40% of unscarified seeds. Scarification of *P. alpina* seeds increased the rate of germination. Unscarified seeds are stimulated by darkness; in scarified seeds there seems to be light-insensitivity.

(3) Unscarified *Poa pratensis* seeds had a higher optimum germination temperature (25° C) and lower percentage of seeds germinated at all temperatures up to the optimum than the two native species. Gentle scarification caused similar results in *P. pratensis* as in *P. alpina*: (1) there was a great increase in percentage germination; (2) a broadening of the optimum temperature range to 10° - 20° C from 25° C for untreated seeds. Both scarified and unscarified seeds do better in darkness.

Scarified seeds of *P. alpina* were the only ones able to germinate under the "cold" regime of 10° C day/5° night (Table II-1) and the percentage germination was 20% lower than *P. alpina* seeds germinated at constant 10° C under continuous light or dark. Only scarified seeds of *P. alpina* and *P. pratensis* were not inhibited by the "warm" regime of 25° C day/5° C night; in fact the germination of the treated seeds was stimulated by this treatment. All other seed populations did less well under this regime than under constant 25° C and continuous light or darkness.

Germination in the three species was rated, based on the length of time required (1) to reach the maximum percentage germination, and (2) for the emergence of the radicle from the seed coat; these data are given in Table II-3. At all constant temperatures and light conditions, it took less than ten days for seeds of *Trisetum spicatum* to reach the maximum percent germination; and in most of the tests, the radicles had emerged within four days of the start of the test. Scarification did not speed up the germination process in this species. For seeds of *P. alpina*, the maximum percentage germinated was not reached for seven to fourteen days; germination occurred more quickly in the light than in the dark. The temperature optimum for germination is not the same as the optimum for rate of germination: the same percentage of seeds germinated at 15° and 20° C but the time required to reach the maximum percentage germinated was shorter at 20° C. Scarification of *P. alpina* seeds decreased the length of time required to reach maximum percentage germination especially for the seeds germinated in the dark. It also greatly decreased the time required for radicle emergence. The seeds of *P. pratensis* took the longest time to reach maximum germination percentage (9.5 to 14 days). Again, scarification increased the rate of germination, particularly in the dark. A decrease in the time to radicle emergence was also observed. The time to maximum percentage germination and radicle emergence in all species except *P. pratensis* (scarified) was shorter at 25°/5° C. Germination occurred most rapidly in unscarified seeds of *T. spicatum* and *P. alpina* (scarified) in 10% of the experiments; in *P. pratensis* (scarified), in 11.25% of the experiments, and in *P. alpina* (unscarified), 1.25% of the experiments. Unscarified *P. pratensis* seeds had more rapid germination than the others only at 25°/5° C.

Table II-3. Number of days to reach maximum % germination, and number of days to emergence of radicle from seed coat.

	Photoperiod	24 hours				0 hours				16 hours	
	Temperature	10°	15°	20°	25°	10°	15°	20°	25°	10°/5°	25°/5°
Poa alpina	Days to max. % germination	13.5	13.5	7.0	8.0	14.0	11.0	11.0	11.0	----a	13.0
	Days to emergence of radicle	8.6	4.5	5.0	3.0	8.6	4.5	7.0	4.0	----a	7.0
P. alpina (hulled)	Days to max. % germination	12.5	10.0	8.5	7.0	10.5	10.5	11.0	4.5	14.0	9.0
	Days to emergence of radicle	4.5	3.0	3.5	2.0	5.5	3.0	4.0	2.0	6.0	3.0
Poa pratensis	Days to max. % germination	13.5	14.0	13.5	9.5	14.0	13.5	13.0	8.0	----a	6.0
	Days to emergence of radicle	5.0	8.0	8.0	4.0	5.0	5.0	8.5	2.5	----a	3.0
P. pratensis (hulled)	Days to max. % germination	12.5	10.0	8.5	9.0	10.5	10.5	11.0	4.0	----a	12.0
	Days to emergence of radicle	4.5	3.0	3.5	2.0	5.5	3.0	4.0	2.0	----a	4.0
Trisetum spicatum	Days to max. % germination	10.0	10.0	7.0	9.0	7.0	9.0	8.0	9.0	----a	14.0
	Days to emergence of radicle	4.0	8.0	4.0	4.0	5.0	8.0	5.0	3.0	----a	7.0

Underlined numbers represent optimal values for that treatment.

a = No germination occurred under these conditions.

Root and shoot lengths measured 14 days after the start of the experiment are given in Table II-4. In general, root growth decreased when the seeds were germinated in the dark, and shoot growth was stimulated. Seedlings of *T. spicatum* grew best at 20° C in both light and darkness; those of both *Poa* species grew best at 15°-20° C in darkness and at 20°-25° C in continuous light. The species with the greatest amount of root and shoot growth at the end of the 14 day experimental period were: *T. spicatum* and *P. pratensis* (scarified), followed by *P. alpina* (scarified), *P. alpina* (unscarified), and lastly *P. pratensis* (unscarified). The seeds germinated under the 16 hour photoperiod and diurnal temperature regime did not do as well as those from the constant temperature regimes. The seedlings that developed from the scarified seeds of *P. pratensis* were very small; and the growth of all the seedlings at 25°/5° except from *P. pratensis* (scarified), was less than at constant 25° C.

P. alpina seedlings, from both scarified and unscarified seeds, tend to have the greatest root:shoot ratios (Table II-5) at all temperatures; followed by *T. spicatum* and then *P. pratensis*. Seedlings from scarified seeds have lower root:shoot ratios than those from unscarified seeds. Root:shoot ratios of seedlings grown at 25°/5° were greater than the corresponding ratios at 25° C in the dark, and for both *T. spicatum* and *P. pratensis* (scarified), comparable to the ratios in the light.

2.4 Discussion

Temperature and moisture are the most critical factors in seed germination, and germination should occur at a time when there is a favorable combination of these factors (Mayer and Poljakoff-Mayber 1963). This does not usually occur at the time the seeds are shed. Low temperature is considered to be the greatest limiting factor to plant survival and growth in the alpine (Clebsch and Billings 1976). Given this, it would be expected that alpine species would have low optimum temperatures for germination, such that germination could be initiated early in the growing season. The results of these

Table II-4. Root and shoot lengths (mean ± standard deviation)* measured after 14 days under various temperature and light conditions.

Species	Photoperiod....	24 hours			
	Temperature....	10°	15°	20°	25°
Poa alpina	Root length (mm)	3.21±1.71	23.61±1.98	47.52±25.46	23.77±3.94
	Shoot length (mm)	0.88±0.18	7.65±1.48	9.51± 1.73	30.51±11.83
P. alpina (*scarified*)	Root	2.94±0.45	25.11±12.35	35.51±14.23	30.15±17.5
	Shoot	1.71±0.52	6.87±1.25	7.47± 2.27	7.70± 3.04
Poa pratensis	Root	0.25±0.02	20.24±2.67	27.72±11.04	58.24±20.68
	Shoot	1.12±0.02	9.97±0.93	14.58± 4.74	16.50± 6.06
P. pratensis (*scarified*)	Root	1.36±0.82	9.11±0.16	21.42±10.42	11.07± 2.62
	Shoot	1.01±0.13	5.34±0.35	7.86± 2.25	12.92± 9.37
Trisetum spicatum	Root	12.09±2.97	9.12±2.95	48.06±15.56	34.80± 11.34
	Shoot	7.61±2.19	4.80±2.12	16.20± 3.10	13.93± 4.07

Table II-4. (Continued)

Species	Photoperiod	0 h				16 h	
	Temperature	10°	15°	20°	25°	10/5°	25/5°
P. alpina	Root	5.93±2.08	22.76±7.10	23.77 ±3.94	12.75±4.64	---a	14.24±8.34
	Shoot	4.88±2.64	27.08±5.34	30.51±11.83		---a	4.48±2.67
P. alpina (scarified)	Root	10.93±2.84	11.86±3.64	13.68± 4.78	8.75±5.07	0.65±0.32	18.26±8.12
	Shoot	8.87±1.09	14.77±4.10	14.97± 7.79	9.35±6.00	0.67±0.25	6.03±2.12
P. pratensis	Root	2.00±0.02	19.29±3.55	21.52±10.45	11.73±7.58	---a	8.58±3.94
	Shoot	1.00±0.02	23.02±11.18	37.36±13.31	35.63±8.12	---a	3.92±0.97
P. pratensis (scarified)	Root	6.57±3.75	13.83±4.29	17.24± 9.28	---b	---a	8.36±3.37
	Shoot	5.52±3.88	16.60±0.40	27.46±11.93	---b	---a	4.30±2.37
T. spicatum	Root	14.04±3.54	15.24±3.47	15.55± 3.82	14.99±4.59	---a	7.18±2.75
	Shoot	11.48±4.70	17.56±3.29	21.70± 1.44	21.58±4.05	---a	4.02±1.51

Table II-5. Ratios of root length (mm)/shoot length (mm) measured after 14 days under various temperature and light conditions.

Species	Photoperiod									
	24 hours				0 hours				16 hours	
	Temperature				Temperature				Temperature	
	10°	15°	20°	25°	10°	15°	20°	25°	10°/5°	25°/5°
Poa alpina	4	3	4	5	1	<1	<1	<1	--9	3
P. alpina (scarified)	1.5	3.5	4.5	4	1	<1	<1	1	1	3
Poa pratensis	<1	2	2	3.5	2	<1	<1	1	--a	2
P. pratensis (scarified)	1	1.5	2	1	1	<1	<1	--b	--a	2
Trisetum spicatum	1.5	2	2.5	2.5	1	1	<1	<1	--a	2

experiments indicate that the unscarified seeds of the native alpine grasses have a relatively high temperature optimum for germination (15°-20° C). Similar values for optimum germination of other alpine species have been reported by Amen (1966), Bliss (1959), and Sayers and Ward (1966). The reason for these rather high temperature optima is revealed in Sorenson's detailed study of the germination process in the arctic tundra (1941). Sorenson found, as have other researchers since, (Sayers and Ward 1966, Billings 1973) that moisture levels are highest in the early summer immediately following snowmelt, and decrease as the growing season progresses, such that there is often a drought in the late summer. The average soil temperatures in early summer are approximately 10°-15° C and increase to their highest values in mid-late summer, and then decrease in the late summer and early fall (Sorenson 1941). The pattern in alpine regions would be similar, and therefore the optimum time for seed germination, in order to maximize the length of the growing season, would be early summer, following snowmelt. Warmer temperatures would be available in mid-summer, but seedlings that germinated this late in the season would face the risk of being caught by the late-season drought, and by the onset of winter frosts before they would be mature enough to survive. Sorenson found that most species germinate in the early summer when soil temperatures are about 15° C, and before the soil dries out in late summer. Although germination is possible earlier in the summer, the probability of survival is decreased at this time, as the seedling is then exposed to the danger of injury from late frosts. Germination does not occur in the late summer, when the seeds are produced , as soil moisture is limited, even though the temperatures are favorable.

This is one way in which alpine plant species have adapted to the selection pressures imposed by the alpine environment. The danger in introducing and attempting to propagate non-alpine species in this environment is that their physiological activity is not synchronized with the habitat, especially with respect to the temperature regime. As illustrated in Fig. II-2, if a species from a warmer habitat is introduced to higher elevations it may retain its tendency to delay development until the temperature has risen considerably; as a result, it germinates too late in the season,and the new growth has no time to mature before the onset of winter. Thus it may be caught by frost and damaged (Larcher 1980). The optimum temperature for unscarified seeds of *P. pratensis* was 25° C. At temperatures up to this optiumum, this introduced species had a lower germination rate than the two native species. It is possible that due to a combination of a higher optimum germination temperature and a slower germination rate, unscarified seeds of *P. pratensis* would germinate too late in the alpine growing season for the seedlings to mature by winter, as described above.

In both *Poa* species, dormancy seems to be environmentally imposed; there was no evidence for another type of dormancy besides that of seed-coat inhibition. Both percentage germination and the rate of germination were increased by gently scarifying the seeds. Scarification also enabled low temperature germination. The enhancement of germination by scarification has been noted by several researchers (Amen 1966, Amen and Bonde 1964, Billings 1974, Bliss 1959). While scarification is an important experimental technique which stimulates germination in many alpine species, it is uncertain how scarification would occur in nature and thus its importance as an adaptation is debatable. It has been suggested that scarification is due to the abrasive action of strong winds (which can create turbulent pockets of sand and gravel) and solifluction (Marr 1961). Both occur commonly in the alpine region, especially on open sites. An advantage of scarification could be to spread germination over an extended period of time so that the process could potentially encompass several favorable periods (Amen 1966). In addition, the seed coat would be destroyed at different rates by different site conditions. Both strategies would prevent simultaneous germination of all the produced seeds, which could result in high mortality rates if conditions became unfavorable.

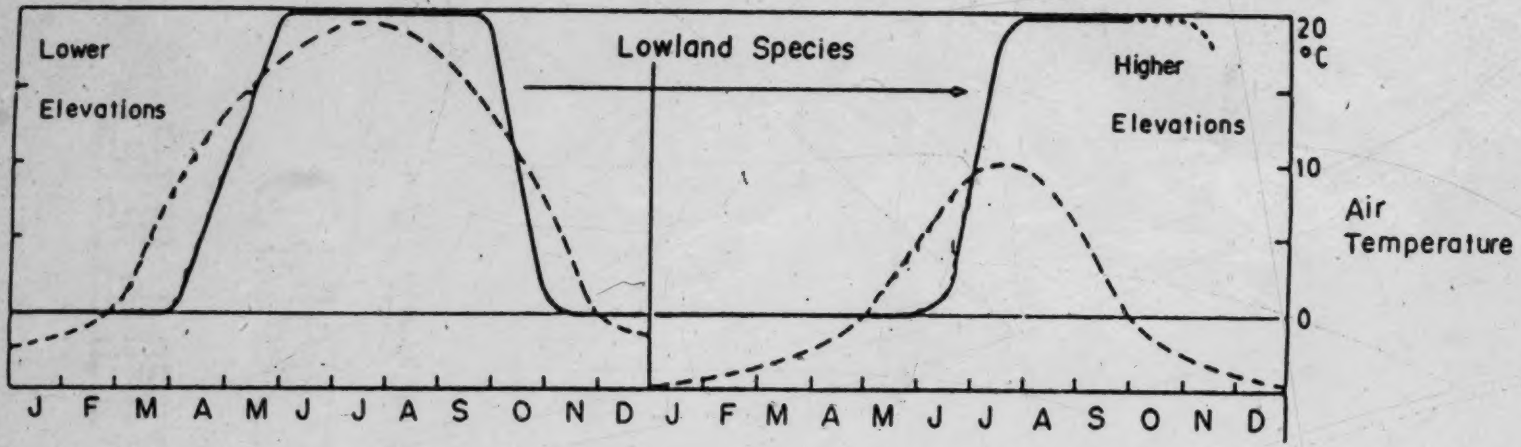

Fig. II-2. Diagram illustrating the temporal coordination of the climatic rhythm and the rhythm of plant growth. The left half of the diagram shows a general synchronization between progression of temperature through the year (dashed curve) and physiological activity (solid curve); in the right half the consequences of lack of adaptation are indicated. Adapted from Larcher, 1980.

Alpine areas are characterized by strong diurnal temperature fluctuations, therefore it would not be realistic to limit germination studies to constant temperatures (Clebsch and Billings 1976). In this experiment two diurnally fluctuating regimes were used; the "cool" regime totally inhibited germination in all but the scarified seeds of *P. alpina*, and even this species had 20% less seeds germinate than at constant 10° C. The "warm" regime conditions, compared with the results from constant 25° C, stimulated the percentage germination in scarified seeds of both *Poa* species, but inhibited germination in unscarified *Poa* seeds and *T. spicatum*. The data presented here are insufficient to support any conclusions drawn about this difference, as the effect of photoperiod was not investigated.

The data indicate that exposure to continuous darkness promotes germination of all three species; this trend is more pronounced in unscarified seeds, and at low temperatures in the native species. Bliss (1959) found that the seeds of most of the 26 alpine species he studied showed no significant reduction of germination in the dark, and greater than one-third showed an increase. Both *T. spicatum* and *P. alpina* seeds fell into this later category, although only *T. spicatum* germination increased significantly (by 23%). Dark stimulation of *T. spicatum* seed germination was also found by Sayers and Ward (1966); they studied seed collected from the alpine zone of Rocky Mountain National Park, Colorado. Clebsch and Billings (1976) studied germination of seeds from several populations of *T. spicatum* collected from 70°N to 41°N in North America; their results indicate that germination is dark-stimulated in the populations from the Rocky Mountains of Canada and the U.S., while the high-latitude populations have light-stimulated germination. This trend was clinal. Ecologically, the increase in germination by darkness may be due to the fact that seeds may commonly be buried by wind-blown sand or silt (Bliss 1959). In *P. alpina*, and to a lesser extent in *P. pratensis* and *T. spicatum*, the dark stimulation can be overcome by scarifying the seeds.

Although darkness stimulates germination, it has a negative effect on root:shoot ratios (Tables II-4, II-5). This result is because the seedlings in the dark are etiolated: stem elongation is stimulated, and root growth is inhibited (Salisbury and Ross 1978). If it is important for seedlings to rapidly develop a root system,as is the case in reclamation where it is necessary to prevent erosion, then the advantages gained in rapid germination would be outweighed by the poor root development. A solution would be to transfer the seedlings to the light as soon as the majority of seeds have germinated.

The rapidity of seed germination of the species studied is an important factor in plant establishment. Although vegetative reproduction is very common, and probably the major source of new plants in the alpine, general abundance may also be related to germination success (Sayers and Ward 1966). For reclamation purposes, the seeds that germinate rapidly and have the greatest percentage germinated are most desirable. Of the three species studied, *T. spicatum* has the lowest percentage germination, but is quick to germinate. The time required to reach maximum germination was five to ten days, depending on conditions, and radicles had emerged within three to eight days. However, the maximum percent germinated was only 62% under optimum conditions, although scarification increased this to 78%. In Bliss' study (1959) the minimum time for germination was eight days, the mean 12, and the last germination occurred at 40 days when seeds were germinated in the light. In the dark, the minimum time was eight days, the mean 15, and the last at 22 days; 71% of the seeds germinated. These results are consistent with the present findings. Sayers and Ward (1966) found *T. spicatum* seeds to have relatively slow germination rates; only after 14 days did any condition tested result in 50% or better germination. They concluded that good field germination of this species would occur only with rather extended periods of favorable temperature and moisture. The present findings are slightly different: 40% of *T. spicatum* seeds germinated in about seven days. Such differences occur because there is intraspecific variation with respect to germination and establishment in this species (Clebsch and

Billings 1976). There was a great deal of intraspecific variability with respect to the parameters of germination measure in this experiment; this variation is especially visible in the root and shoot length data (Table II-4).

The minimum time for germination of untreated *Poa alpina* seeds was four days, and it took from seven to fourteen days to reach maximum percentage germination, depending on the conditions. These data agree with Bliss'(1959) data on *P. alpina* germination: in the light, 85.5% germinated, with a minimum time to germination of eight days. In the dark, 91.2% of the seeds germinated and the minimum time to germinattion decreased to five days. In the present experiment, the scarified seeds took less time to germinate and reached maximum percent germination sooner. At temperatures above 10°C, both untreated and treated seeds of *P. alpina* had greater percentage germination than those of *T. spicatum*, and scarified seeds germinated as rapidly as those of *T. spicatum*.

Poa pratensis seeds took the longest amount of time to start germinating (five to nine days), and did not reach peak germination before 14 days. Again, scarified seeds reached peak germination earlier than unscarified seeds, and reached maximum percentage germination slightly earlier. At all temperatures up to 25° C unscarified and scarified seeds of *P. pratensis* had lower percentage germination than similarly treated seeds of *P. alpina*. *P. pratensis* also had lower germination percentages than *T. spicatum* up to 25° C unless the Nugget seeds were scarified. Better percentage germination in unscarified seeds of this species might be obtained by exposing the seeds to greater diurnal temperature fluctuations. Thompson *et al.* (1977) found that *P. pratensis* and other sward-invading species required temperature fluctuations of about 10° C for maximum germination. This requirement is related to the grasses' ecological role as gap-invaders.

As mentioned above, favorable conditions for seed germination in the alpine may exist for only a short time; thus *Trisetum spicatum* has an advantage over the other species due to its rapid germination rate. However, the population of *T. spicatum* seeds used in this experiment had lower germinability than the other two species; other populations of *T. spicatum* have been shown to have greater percent establishment (Clebsch and Billings 1976). For both species of *Poa* both rate and percent establishment are greatly increased once the seeds are scarified; both processes are increased to a greater extent in *P. alpina*. *P. alpina* also has an advantage in terms of early seedling development; although *T. spicatum* seedlings are faster growing, *P. alpina* seedlings have greater root length than shoot length after two weeks of growth.

These observations have important implications for the use of the species in reclamation. Plummer (1943) found that the most successful species of the 12 range grasses that he studied were those with greater root growth in the seedling stage. Not only would this enable *P. alpina* seedlings to become established and productive earlier than those of the other two species, but the seedlings would have a greater area for storage of carbohydrates, meaning that the seedlings would have a better chance for surviving unfavorable periods of drought or frost. In addition, the greater the root growth into spoils, the greater the soil stabilization and the greater the enhancement of soil developmental processes of disturbe~~d sites. Las~~tly, Nicholas and McGinnies (1982) found that three to four years were required before root systems of plants grown in mine spoils resembled those grown in natural soils in terms of weight and distribution. On such disturbed sites where natural growth is retarded, it is a definite advantage to a plant species to have rapid and abundant belowground development.

Given the germination characteristics of *Poa alpina*, *P. pratensis*, and *Trisetum spicatum*, these species could all be utilized in a direct-seeding program, with best results using scarified seeds.

2.5 Conclusions

These observations suggest that for reclamation of high-elevation disturbed sites, *P. alpina* would be most successful as it has high percentage germination at 15° C and does not need to be scarified to give reasonable germination rates, although scarification increases germination rate and enables lower temperature germination. In addition, the seedlings of *P. alpina* have the highest root:shoot ratios of the three species studied here. *T. spicatum* seeds have a faster germination rate, but have lower percentage germination and smaller root growth in terms of length.

This set of experiments was intended to determine whether indigenous alpine and introduced grass species possess different germination characteristics that would influence their success in revegetation of alpine disturbances. The results indicate that, based on percentage germination, rate of germination, and root:shoot ratios, *P. alpina*, followed by *T. spicatum* would be more successful than *P. pratensis* for alpine reclamation. However, these germination tests were all carried out in controlled growth chambers, using Petri plates and filter paper; these conditions are stable in time, spatially uniform, and free of biotic interactions (Evans 1963). In their natural environment, these seeds would be exposed to constantly changing conditions, a range of diverse microclimates, and numerous other organisms. It is not possible to completely predict field performance based on these results, since several major factors such as soil fertility and pH that greatly influence plant survival and performance were not studied.

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3. Temperature Resistance

3.1 Introduction

Freezing injury is a major cause of plant death, especially in crop plants, and low temperature is reputedly the single most important factor in natural plant distribution (Parker 1963, Burke *et al.* 1976). For these reasons, plant resistance to low temperatures has been extensively studied. In contrast, there have been far fewer studies dealing with heat resistance in plants, despite the fact that there is a limiting high-temperature stress for all organisms (Levitt 1980). Most studies have concentrated on three aspects of the relationship between plants and temperatures: (1) the nature of the temperature stress; (2) injury resulting from exposure to temperature extremes; and (3) mechanisms of temperature resistance.

3.1.1 The Freezing Process

Freezing in plants is the conversion of liquids in cells and tissues to a solid state with the loss of heat (Alden and Hermann 1971). Two types of freezing are commonly differentiated: intracellular and extracellular, based on the location of ice formation in the tissue and the rate of freezing. Intracellular freezing occurs when plant tissue is very rapidly frozen, causing thousands of ice crystals to form in the protoplasts and vacuoles (Burke *et al.* 1976). This type of freezing is readily induced by artificial conditions, but rarely occurs under natural climatic conditions as it requires a very rapid and substantial decrease in temperature. Extracellular freezing is much more common in nature. For this reason, our research has been mainly concerned with extracellular freezing.

The process of extracellular freezing can be summarized as follows (after Levitt 1980 and Rowe 1967): when plant tissue is cooled slowly (i.e. at a rate of about 3° to 5° C per hour), ice normally crystallizes in the large xylem vessels, since their large diameter doesn't allow for supercooling and their sap is dilute. From these nucleation points, freezing proceeds along the xylem through the plant body. The plasma membranes prevent ice crystals from innoculating cell contents and thus the ice spreads via intercelluar spaces, at the expense of the water vapor in the spaces and surface water film on the cell walls. As the temperature of the tissue decreases, supercooling occurs in the cells due to their relatively small size and because their membranes have prevented intracellular ice formation. The phenomenon of supercooling occurs because water does not necessarily solidify when its temperature is at the freezing point. Molecules must be arranged in a regular manner to form a crystalline matrix; when the freezing point is reached it may take time before the necessary pattern forms, and until this time the water remains liquid (Boikess and Edelson 1981).

As the tissue temperature drops below the freezing point, the vapor pressure in the cell is higher than that outside the cell, since the formation of ice causes the vapor pressure of the intercellular spaces to drop sharply. Thus the diffusion of water through the plasma membranes to the intercellular spaces is favored. This process results in ice growth outside the cell and in dehydration of the cell protoplast. At some supercooling point, ice begins to crystallize, and the heat of fusion is released. The maximum temperature attained by this exotherm is the tissue freezing point (Fig. III-1). Extracellular freezing is a slow gradual process; often it is slow enough so that ice does not spread throughout the plant, but only forms in certain areas.

When thawed, plant tissues are usually limp and appear waterlogged. As ice in the intercellular spaces melts, the spaces are left almost free of air, and the cells are flaccid due to the water lost to ice (Levitt 1980). If the tissues are uninjured, water is readily reabsorbed by cells. Injured cells cannot reabsorb water and thus are characterized by "frost plasmolysis" (Levitt 1980).

Page 59 has been removed because of the unavailability of copyright permission. Page 59 contained information on a typical curve of slow controlled rate of cooling (from Rowe, 1967).

3.1.2 Freezing Resistance

The ability of some plants to survive subfreezing temperatures is due to (1) the ability of the protoplasm to tolerate low temperatures and sudden cell dehydration, *i.e.* tolerance mechanisms; or (2) the effectiveness of mechanisms aimed at delaying injury caused by exposure to cold and dehydration, *i.e.* avoidance mechanisms (Levitt 1980). In some plants, frost resistance is due to a combination of both tolerance and avoidance mechanisms. Tolerance mechanisms are of long-term duration, and the level of tolerance changes throughout the year: it is lowest in the summer, increases through autumn to reach a maximum level in the winter and decreases in the spring (Larcher 1962). In contrast, avoidance mechanisms are of shorter duration; one of the most important is supercooling (the ability to cool to temperatures below the freezing point without ice formation as described above). Although supercooling can only be maintained for a few hours, it can provide a reliable degree of protection against frosts of short duration (Larcher 1973, Levitt 1966, 1980). This protection is especially important against frosts occurring during the summer, when tolerance levels are at their lowest. As mentioned earlier (see 'General Introduction'), temperature resistance is a major factor in the persistence of a certain species in any particular region (Larcher 1973). In the alpine the growing seasons are short, cold, and unpredictable; and outside the arctic, they are also characterized by cold nights (Billings 1974a and b). Thus plants growing in the mountains face a risk of short-duration frosts at anytime during the growing season. For these plants, avoidance mechanisms are an efficient means of surviving the frequent cold spells and frosts.

It might therefore be expected that the native grass species would be better adapted to survive low temperature stress than *P. pratensis* which is not an alpine species. Secondly, given the origin of the *Poa alpina* populations, it was expected that *P. alpina* from (elev. 2000m) would have the greatest frost avoidance, as it is from the valley bottom of Fairview Mountain, Alberta. Fliri (1962) found that high elevation sites have more frost days (when the minimum temperature is below zero) and ice days (when the maximum temperature is below zero) than valley sites (Table III-1). However, at 500 metres there are more frost-exchange days (when the temperature crosses 0° C) than at 3000 metres; this fluctuation in temperature over a relatively short interval of time causes more stress for plants than does the consistently below-zero temperatures of higher elevations. Thus, at the lower elevations, a trend towards avoidance mechanisms would be expected, to survive the constantly changing conditions; at very high elevations, tolerance mechanisms would be more effective. As well, temperature inversions occur frequently in some valley bottoms when cold heavier air from higher up the mountains moves downslope and, if prevented from draining down out of the valley by an obstacle such as a hill or forest, accumulates in the valley. Ogilvie (1969) found that some valley bottoms could be 10°-20° C cooler than sites further up the mountain, and inversions were possible almost every day of the month. For these reasons some valley bottom plant commumities are composed of low-temperature-enduring plants.

Thus the first objective of this study was to answer the questions (1) at what temperature do the three grass species freeze?"; and (2) "are they able to survive this stress?". To answer these questions, the frost avoidance of these species was determined by studying their supercooling and freezing points. Frost avoidance was chosen as it is easier to measure accurately than tolerance. This information will reveal whether any one of the three grasses is better able to survive the periodic frosts occurring in open disturbed alpine sites.

3.1.3 Heat Resistance

For many plant species, dangerously high temperatures can occur under natural conditions: the temperature of leaves exposed to sunlight may be well above that of the air, due to absorption of 44% - 88% of the total radiation received by the leaves (Raschke 1960). As for frost resistance, the ability of a plant to survive high

Table III-1. Temperature data from the European Alps.

Elevation	Number of FROST-DAYS (Min<0°C)	Number of ICE-DAYS (Max<0°C)	Number of "FROST-EXCHANGE" DAYS (F-I)
500 m	122	33	89
3000 m	305	255	80

Adapted from Fliri 1962.

temperatures is due to both avoidance and tolerance mechanisms. Seasonal changes occur in heat tolerance as in frost tolerance, and actually the level of heat tolerance increases with frost tolerance in the winter (Santarius 1973, Palta *et al.* 1978), thus there must be a common mechanism operating at this time. However, Santarius (1973) also found that heat tolerance increases in the summer, so there must be a second mechanism that raises heat tolerance, different from the first. Heat tolerance differs from cold tolerance in that the seasonal changes are very small, i.e. less than 5° C, and as mentioned above, the level of heat tolerance does not follow the climatic rhythm (Larcher 1973). Heat tolerance levels can change within a short period of time; Alexandrov and Yaskuliev (1961) found a rise in tolerance from morning to afternoon in a species of desert grass.

Mechanisms of heat avoidance include (1) transpirational cooling, (2) insulation, and (3) decreasing the absorption of radiation. An example of this last mechanism is found in members of the Fabaceae, which are able to reorient their leaves such that less surface area is directly exposed to incoming solar radiation, while keeping transpiration fully functional (Walter 1979). Unlike the situation for frost avoidance, heat avoidance is very difficult to measure. Heat tolerance is easier to measure with a high degree of precision (Larcher 1973). Either tolerance of indirect effects can be measured by measuring the temperature response of a specific process, such as photosynthesis, or the tolerance of direct effects can be measured, as was done in this experiment, by exposing the plants to high temperatures and monitoring the results.

For many reasons high temperatures are a limiting factor for plant establishment on alpine disturbances. Even on undisturbed sites soil temperatures can be extremely high: Turner (1970) measured soil temperatures of greater than 80° C at 2000 metres. As mentioned in the General Introduction, soil temperatures on disturbed sites in the alpine can reach temperatures as high as 50°-75° C. High temperatures occur in the plants themselves when irradiation is very strong as is certainly the case on such open sites. Leaf temperatures of grass species growing on Mountain Park mine spoils in July of 1984 commonly reached 40° C (Fig. I-6, General Introduction). Furthermore, when soil is exposed to high irradiation, heat damage or killing of the plant tissues at ground level occurs (Levitt 1980, Schramm 1966). High temperatures also limit plant growth and establishment as they cause more rapid depletion of vital storage compounds (Johnson 1980). Thus it is important to have a measure of the relative ability of the three grass species to resist and survive high temperatures, in order to assess their suitability to reclaim such areas. The second objective of this study was to answer the question "What is the highest temperature these grass species can survive?".

3.1.4 Viability

For both frost and heat resistance in order to determine if the plant could survive the temperature treatment some measurement of viability had to be made. In theory, viability is "...the ability to live, grow and develop."(Palta *et al.* 1978). Most viability tests are able to measure only if tissue is living or nonliving because of certain complications: (1) in a multicellular organism or tissue not all cells die simultaneously; (2) within a cell, different organelles cease functioning at different times; and (3) in order to be truly useful, the test should determine how well the treated cell, tissue, or organism will continue to fulfill its life functions in relation to a control (Palta *et al.* 1978) The TTC (2-3-5-triphenyltetrazolium chloride) reduction method is commonly used to evaluate viability, and the modified method of Steponkus *et al.* (1967) was used early on in these experiments. However, there was great variability among the replicates; this problem has been encountered by other researchers (see Stergios *et al.* 1973). Thus for these experiments, the plants' ability to resume normal growth after temperature treatment was used as the measure of viability. This method has been suggested as the ultimate criterion of viability by Palta *et al.* (1978).

3.2 Materials and Methods

All plants of *Poa alpina* used in these experiments were provided by the Vegetation Group working with native grasses at the Alberta Environmental Center in Vegreville, Alberta. A description of the origin and method of collection of these plants is given in the General Introduction. Plants of *Trisetum spicatum* and *Poa pratensis* were grown from seed in controlled environment chambers at the University of Alberta. Seed of *T. spicatum* was provided by the Alberta Environmental Center; *P. pratensis* seed was provided by Prairie Seed Company. Plants were not used for these experiments until they had been growing in the growth chambers for about two months.

3.2.1 Frost Resistance

3.2.1.1 Freezing-point Determination

Several previous studies (e.g. Pisek, Larcher, and Unterholzner 1967, Gerber and Hashemi 1965) have compared the freezing points obtained using both detached and intact leaves, and have found no significant difference in the freezing points obtained. Therefore, for these experiments, detached leaves were used to determine the freezing and supercooling points of the three grass species. The method of Pisek *et al.* (1967) was followed, in which copper-constantan thermocouples were enfolded inside detached leaves; foam rubber insulation was then placed around the leaf and secured with elastic bands, which also helped hold the thermocouple in place (Fig. III-2a). This technique provides insulation so that the heat produced when ice forms will not dissipate into the surrounding air. A thermocouple was also placed in an insulated vial of pure water, which has a freezing point of 0° C, to verify this method's accuracy.

The leaves were frozen in the center chamber of a domestic freezer with a temperature range of 10° to -20° C; a fan was also placed in the freezer to prevent layering of air and thus uneven cooling of the tissue. The freezer

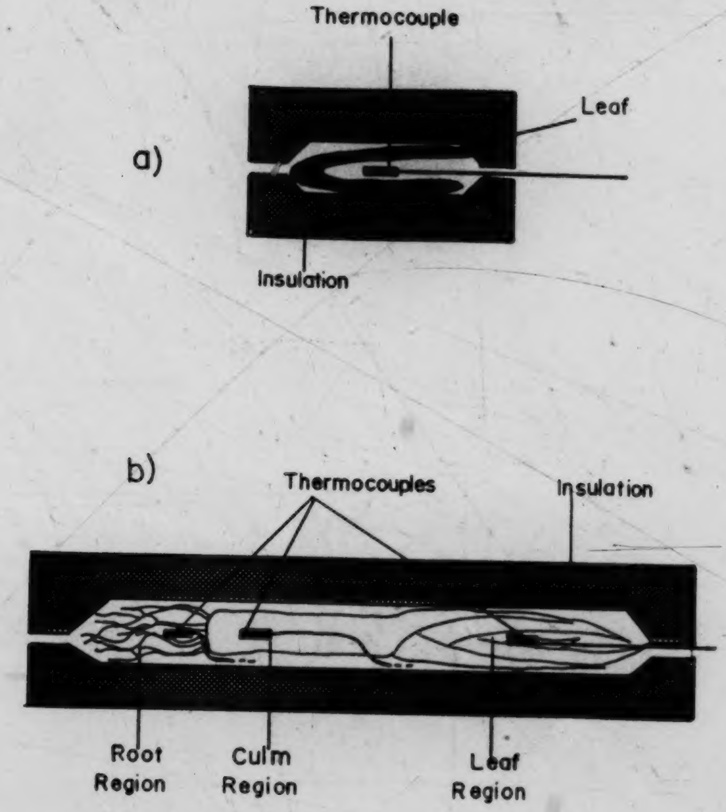

a)
Thermocouple
Leaf
Insulation
b)
Thermocouples
Insulation
Root Region
Culm Region
Leaf Region

Fig. III-2. Apparatus for determining the freezing point of plant material
a) of detached leaves
b) of whole tillers.
See text for explanation.

temperature was slowly decreased at the rate of about 5° C per hour; this slow cooling is standard procedure in most freezing studies, since rate is known to influence freezing point. Freezing of all leaves occurred within six hours. The output of the thermocouples was recorded on a Honeywell multipoint strip chart recorder, which was calibrated using a standard thermometer for the temperature range of 30° to -20° C. Resolution to 0.25° C was easily made on the chart output. The freezing curves produced by this method show a slow steady decline in temperature to the supercooling point, followed by a sharp rise in temperature to the freezing point, as the heat of fusion is released. This method has several advantages: it is simple, allows for several replicates to be obtained within a short period of time, and works well with various plant parts. However, this method did not allow for determination of viability and thus the ability of the plants to resume growth was measured.

3.2.1.2 Determination of Viability

In order to determine viability by re-growth, the method of Pisek *et al.* (1967) was modified using whole tillers instead of detached leaves. Whole plants were divided into individual tillers, which were then placed in test tubes filled with full strength Hoaglands' solution. This procedure did not harm the tillers, as they grew very vigorously in the test-tubes. Before being frozen, the tillers were removed from the test-tubes and blotted to remove excess surface water as this can cause freezing at higher temperatures than natural (Levitt 1980). Three thermocouples were used for each tiller so that we could monitor the temperature of the leaves, the roots, and the culm region. Each plant was wrapped in foam insulation and the thermocouples secured with rubber bands, as above (Fig. III-2b). For each tiller, three freezing points and supercooling points were obtained, corresponding to the three regions.

Injury caused by freezing may be repairable; cool temperatures have been shown to enhance repair whereas room temperatures (i.e. 20° C) inhibit repair (Levitt 1980). Therefore the standard procedure is to keep plants cool (at 0° to 5° C) for 24 hours after thawing (Levitt 1980). Thus after all the tillers had frozen they were removed from the freezer and quickly placed in a refrigerator at 4° C for 24 hours. The individual tillers were then replanted and placed in their original growth chamber. Death of extracellularly frozen plants may not occur for days or longer following thawing (Levitt 1980) so the tillers were observed daily for four weeks. While most damage was visible within one week of treatment as obvious necrosis and shriveling of the leaves, there was the chance of regeneration from belowground parts.

It was observed that the freezing points obtained from detached leaves were significantly different from those obtained from intact leaves. We decided to examine whether separation of the roots, culm and leaves would alter their freezing points. For this experiment, the tillers were divided in the three regions, thermocouples were enfolded as described above, and each section was separately wrapped in insulation.

3.2.1.3 Heat Resistance

For this experiment, the method of Larcher (1962) and Kreeb (1977) was followed. Tillers were obtained as described above. To determine heat resistance, the tillers were placed in a thermos of hot water for 30 minutes. The range of temperatures tested was from 40° to 50° C; there was no need to go higher than 50° C as no plant survived this temperature. As a control, tillers were placed in water at room temperature for thirty minutes. After half an hour, the tillers were removed from the water, replanted into pots, and placed in their original growth chambers. In order to determine viability, they were monitored for four weeks as was done in the freezing experiments.

3.2.2 Results

3.2.2.1 Freezing Resistance

A one-way ANOVA (Table III-2) done on the supercooling temperatures indicated a significant difference between the species, so a GT-2 test (Sokal and Rohlf 1981) was done to determine the specific differences among the means. The results of this test are given in Fig. III-3. There is no significant difference between the *Poa alpina* populations from 2700 metres and 2300 metres at either growth temperature. The *P. alpina* population from 2000 metres was significantly different from the other two populations at both growth regimes. When grown under the warm regime, this population had the lowest supercooling point; under the cool regime, the supercooling point was significantly higher than those of the other two populations. *P. pratensis* from the cool regime supercools at a significantly higher temperature than the other species, with the exception of *P. alpina* from 2000 metres. Its supercooling point when grown under the warm regime is equivalent to that of *P. alpina* and *T. spicatum*. The supercooling temperature of *T. spicatum* does not change significantly from the warm to the cool growth regime; nor is it significantly different from those of the *P. alpina* populations, except for that of the population from 2700 metres, grown at 10°/5° C.

A one-way ANOVA (Table III-3) indicated a significant difference between the freezing points of the grasses; the GT-2 method was again used to illustrate the differences between the means. The average freezing points and 95% comparison intervals for the three grass species are given in Figure III-4. When the plants were grown in a cold regime of 10°day/5°night, there was no significant difference between the three *P. alpina* lines, or any *P. alpina* line and the other two species. There was a significant difference between *T. spicatum* and *P. pratensis*, with *T. spicatum* having the lower freezing point. For the plants grown

Table III-2. One-way ANOVA of the supercooling temperatures of three grass species.

SOURCE	DF	SS	MS	F
Among groups	9	721.21	80.13	14.07**
Error	272	1548.93	5.69	
Total	281	2270.15		

** = $p \leq 0.01$

Fig. III-3. 95% comparison intervals by the GT-2 method for the means of supercooling temperatures of three grass species. Means whose intervals do not overlap are significantly different.

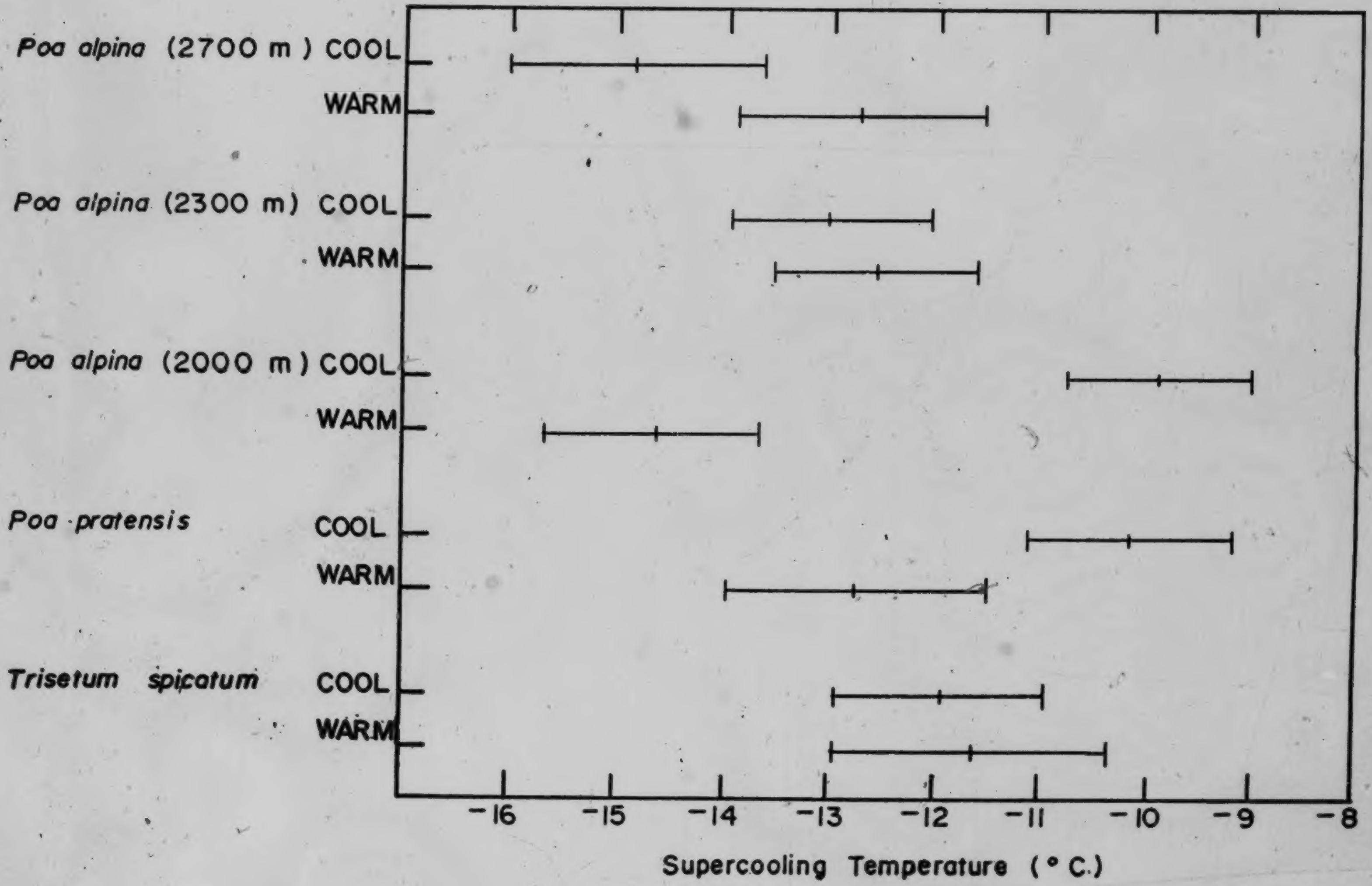
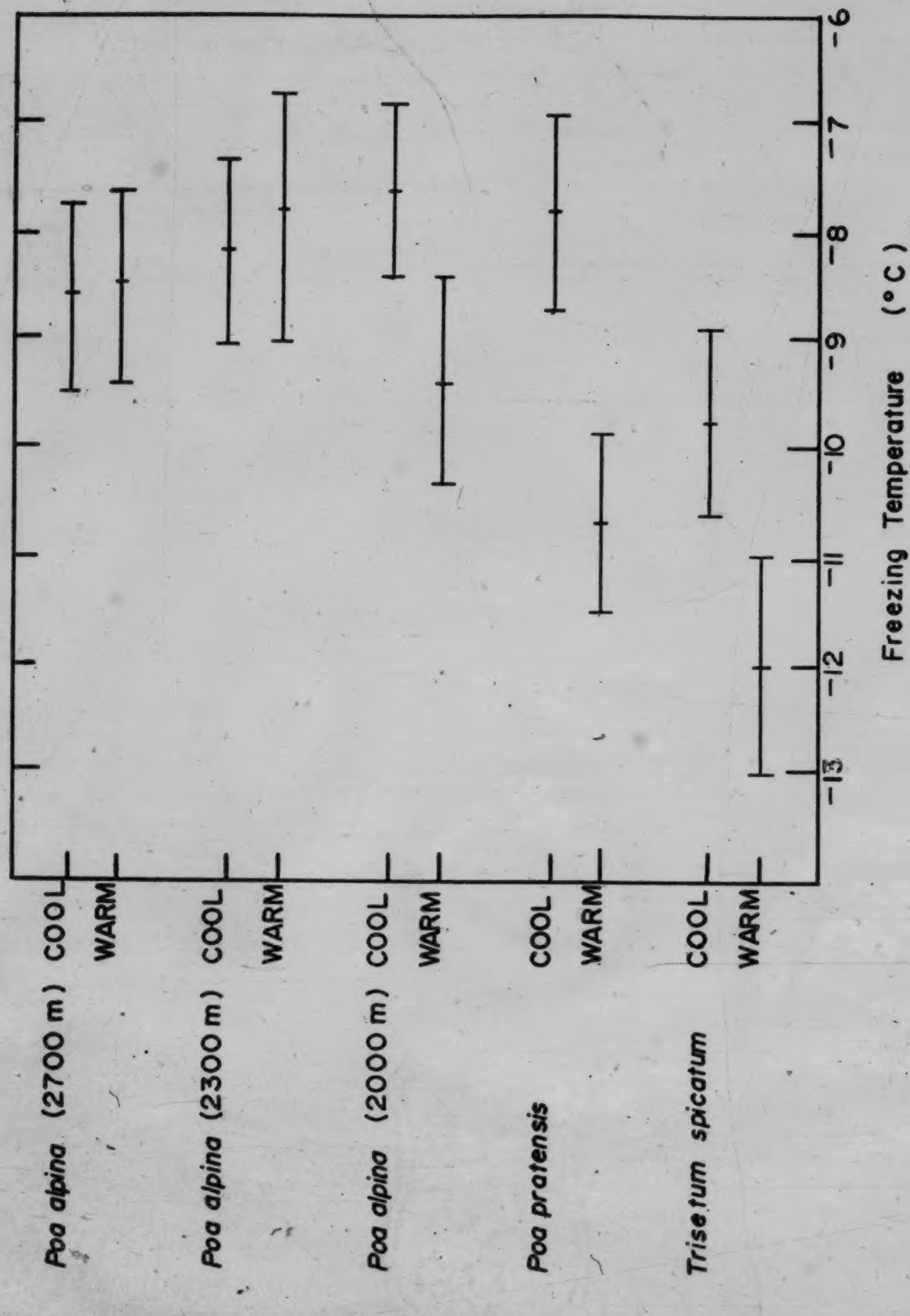

Poa alpina (2700 m) COOL
WARM
Poa alpina (2300 m) COOL
WARM
Poa alpina (2000 m) COOL
WARM
Poa pratensis COOL
WARM
Trisetum spicatum COOL
WARM
−16
−15
−14
−13
−12
−11
−10
−9
−8
Supercooling Temperature (° C.)

Table III-3. One-way ANOVA of the freezing temperatures of three grass species.

Source	DF	SS	MS	F
Among groups	9	923.49	102.61	18.58**
Error	344	1900.14	5.52	
Total	353	2823.63		

** $p \leq 0.01$

Fig. III-4. 95% comparison intervals by the GT-2 method for the means of freezing temperatures of three grass species. Means whose intervals do not overlap are significantly different.


Poa alpina (2700 m) COOL
WARM
Poa alpina (2300 m) COOL
WARM
Poa alpina (2000 m) COOL
WARM
Poa pratensis COOL
WARM
Trisetum spicatum COOL
WARM
−6
−7
−8
−9
−10
−11
−12
−13
Freezing Temperature (°C)

in the warm regime of 25°day/5°night, there was a marked decrease in the freezing points of both *T. spicatum* and *P. alpina*; while this trend was not as marked in the lines of *P. alpina* , the trend towards lower freezing points was present. The freezing and supercooling temperatures, with standard deviations, are summarized in Table III-4.

Figure III-5 and Table III-5 give the results of the freezing point of detached plant parts versus the freezing points of the parts of intact tillers. Separated leaves of *Poa alpina* have a significantly lower freezing point than any other part, including attached leaves. There is no significant difference between the freezing points of any other parts; but there is a trend that the parts freeze in the same order, whether detached or attached : roots freeze first, then the crown region, and lastly, the leaves freeze. Separated roots are the most susceptible to freezing; both leaves and crowns tend to have lower freezing points when separated.

Results from the TTC assays are not included, as the data were extremely variable. The results from the re-growth viability tests show that plants exposed to freezing temperatures in these experiments were always killed, except for one tiller of *P. alpina* which regenerated leaves from underground. (out of about 100 plants used in these experiments).

3.2.2.2 Heat resistance

The temperature at which less than 50% of the exposed plants survive is known as the killing point (Larcher 1980, Steponkus 1984). Table III-6 shows the percentage of plants of the three species that survived exposure to high temperatures. 50° C is a lethal temperature for all three species: not one plant survived exposure to this temperature. For *P. alpina*, 45° C is approximately the killing point. *T. spicatum* is more sensitive to this temperature than the other two species, as less than 50% of the exposed plants survived. *P. pratensis* is less sensitive, as greater than 50% survived 45°C.

Table III-4. Freezing and supercooling points (mean ± standard deviation) of grass species.

Species	Growth Regime	Supercooling Temperature	n	Freezing Temperature	n
Poa alpina (2700 m)	10°/5°	-14.88 ± 2.52	21	-8.63 ± 3.00	42
	25°/5°	-12.76 ± 2.41	22	-8.53 ± 2.13	32
P. alpina (2300 m)	10°/5°	-13.02 ± 2.79	33	-8.22 ± 2.24	40
	25°/5°	-12.60 ± 2.96	33	-7.89 ± 2.23	22
P. alpina (2000 m)	10°/5°	-9.94 ± 2.08	40	-7.62 ± 2.83	38
	25°/5°	-14.66 ± 2.15	31	-9.40 ± 2.40	35
Poa pratensis	10°/5°	-10.20 ± 1.80	33	-7.83 ± 1.74	35
	25°/5°	-12.78 ± 1.94	21	-10.71 ± 2.66	41
Trisetum spicatum	10°/5°	-11.95 ± 1.69	30	-9.77 ± 2.93	40
	25°/5°	-11.66 ± 3.46	18	-12.01 ± 2.02	29

Table III-5. Two-way of ANOVA of freezing temperatures of three portions of *Poa alpina* from three different elevations.

SOURCE	DF	SS	MS	F
Plant part	2	8.285	4.142	7.410**
Elevation	2	0.282	0.141	.242 NS
Interaction	4	5.748	1.437	2.571 NS
Error	27	15.105	0.559	
Total	35	29.420		

** = $\leq$ 0.01

Fig. III-5. 95% comparison intervals by the T-method for the means of freezing temperatures of different parts of *Poa alpina* plants. Means whose intervals do not overlap are significantly different.

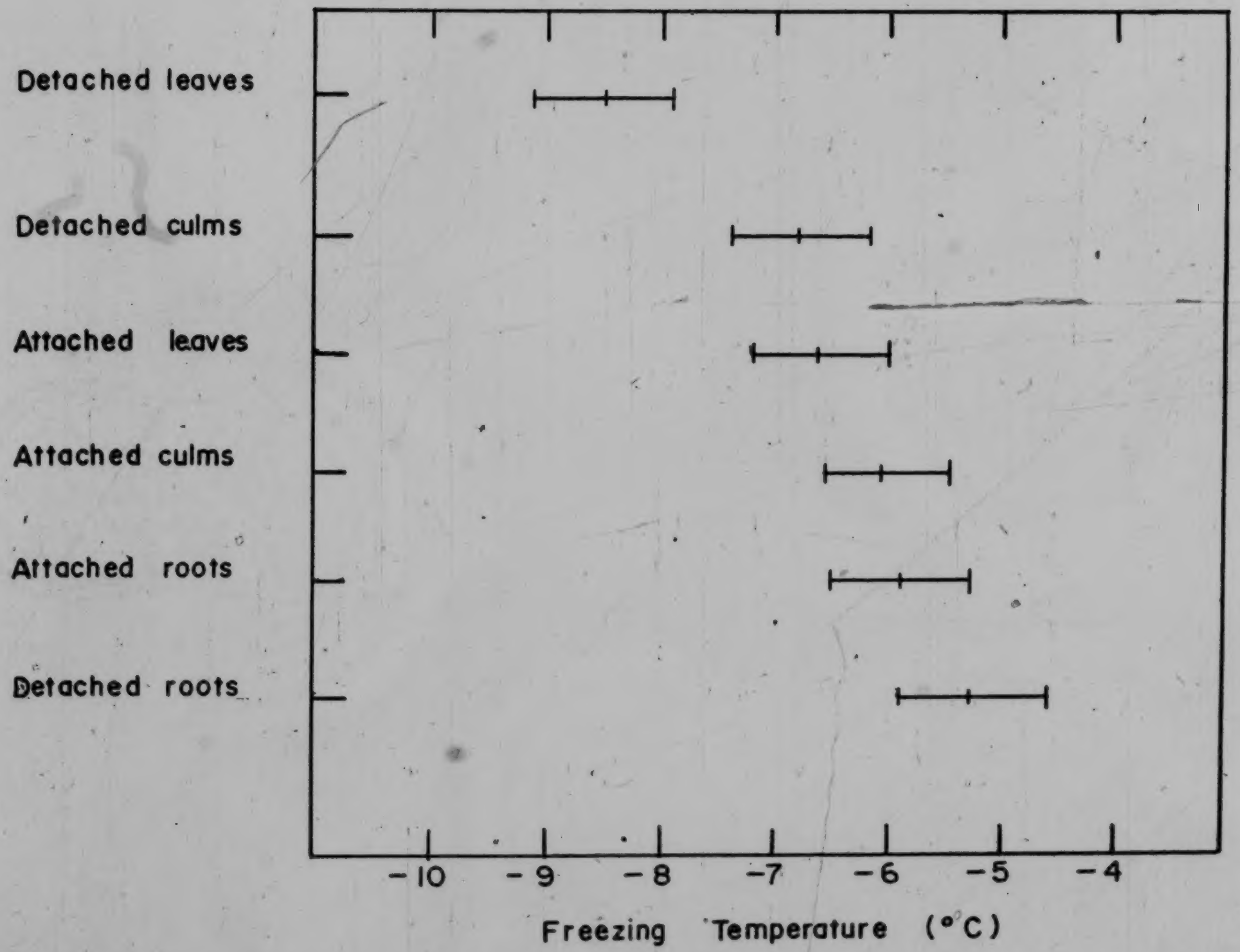

Detached leaves
Detached culms
Attached leaves
Attached culms
Attached roots
Detached roots
-10
-9
-8
-7
-6
-5
-4
Freezing Temperature (°C)

Table III-6. Heat Resistance.

	% plants surviving heat treatment			% surviving	
Species	at 40°C	at 45°C	at 50°C	control (22°C)	n=
Poa alpina	100	47.5	0.0	100	30
Poa pratensis	100	66.5	0.0	98	25
Trisetum spicatum	75	25.0	0.0	90	25

3.3 Discussion

3.3.1 Frost avoidance

On the basis of these data there is limited evidence for agamotypic differentiation in frost avoidance ability between lines of *Poa alpina*.

The lower supercooling point of *P. alpina* from the valley bottom is evidence for greater frost avoidance in this population; the freezing point of this population is also lower than those of the higher elevation populations, although not significantly so. The higher supercooling and freezing temperatures in this population when grown in the cool regime may indicate that this regime is not favorable for its growth, and so the plants would not be able to produce as many cryoprotectants as under the more favorable warm growth regime. The fact that there is no differentiation between the frost avoidance of the lines may indicate that the original temperature regimes of the three sites are not very different, It is also possible that the plants from 2000 metres were not collected from an open area of the valley bottom but from a forested, protected zone. In addition, inversions may not often occur in the Fairview Mountain valley bottom. Climatic data was not available for the area, to determine the frequency of inversions.

Plants growing in alpine areas are exposed to cold stress even during the growing season (Billings 1973, Billings and Mooney 1968, Bliss 1962, Tranquillini 1964). It might be expected therefore that the native alpine grasses would be better adapted to survive low temperature stress than *P. pratensis*, which is not an alpine species. However, there is limited evidence for differentiation in frost avoidance ability between the native and the introduced species. *P. alpina* populations grown in the cool regime (except for the population from 2000 meters) have the advantage of a greater ability to supercool than does *P. pratensis*. When both *Poa* species are grown in the warm regime, there is no significant difference between their supercooling abilities. *P.*

pratensis had the same freezing point as *P. alpina* when grown under the cool regime and a lower freezing point when in the warm regime. This is likely because the variety Nugget has been bred for winter resistance (Elliott and Bolton 1970). Of the three species, *Trisetum spicatum* appears to have the greatest ability to avoid freezing, based on its freezing and supercooling points, whether grown in the cold or warm regime. *P. alpina* from the valley bottom also displays a high degree of frost avoidance, provided it is grown in the warm temperature regime.

The results of the viability tests indicate that for all three species exposure to freezing temperatures is a lethal stress. The causes of death by freezing have been extensively debated.

3.3.2 Freezing Injury

The cooling rate is often considered as the most important factor in freezing damage, but recent studies are challenging the traditional view that warming rate plays only a simple role in plant injury, i.e., the faster the warming rate, the better. Rowe (1967) states that "...for highest viability [warming] needs only to be rapid to prevent ice crystal growth and concomitant cellular damage with prolonged exposure.". These conclusions agree with those of Mazur (1969), who found that slow warming maximizes recrystallization and thus, injury. It is becoming evident that the rate of warming of the tissue plays as important a role in plant injury as does the cooling rate. Levitt (1980) has summarized several studies and concludes that for any hardy plant there will be a critical freezing zone, above and below which the rates of cooling and warming have no effect on survival. In the critical zone, both rates are important. Mazur (1965) proposed that this phenomenon arose from the interaction of two damaging mechanisms, one that operates at slow rates and is associated with solution concentration effects, and another operating at fast rates, causing damage by intracellular ice formation. This theory has been generally accepted, but as recognized by Jacobsen *et al*. (1984), this theory is based on experiments dealing only with single-cell systems; while the theory would apply to a certain extent to organs, as they are composed of cells, organs have thermodynamic and biological properties quite different from those of single cells in suspension. It is expected that optimal cooling and warming rates for organs should be low, for several reasons: the bulk of an organ makes uniform *and* rapid heat transfer impossible (Jacobsen *et al.* 1984); as mentioned above, the location and morphology of ice is affected by cooling rate and less damaging ice structures have been reported with slow rather than rapid cooling; and there is evidence that slow warming is preferable when cells have been slowly cooled (Jacobsen *et al.* 1984, Mazur 1977). In addition, since the water flux out of the internal cells of a tightly-packed cluster will lag behind the water flux out of exterior cells, the amount of water retained in the interior cells of an organ will be greater than that in the exterior cells at a given time and temperature (Levin *et al.* 1977). Therefore there is a greater risk of intracellular ice formation in an organ's interior cells than in a single isolated cell, and ice formation may occur at rates lower than those applicable to individual cells. The results of Levin *et al.* (1977) indicate that only at very slow cooling and warming rates, for which water fluxes are small, can all cells in an organ shrink and swell almost uniformly; at fast rates, the exterior cells expand or contract to a greater degree than interior cells. Jacobsen *et al.* (1984), studying the effect of cooling and warming rates on rabbit kidney survival, found that the best results were obtained using a cooling rate of 1°C/hr and a warming rate of 1°C/minute. The results of a recent study by Hawkins *et al.* (1985) on the viability of renal tissue subjected to a variety of cooling and warming rates indicate that optimum viability occurred with two different combinations of warming and cooling rates. The greatest survival was obtained when the tissue was either cooled at a rate of 3°C per hour and warmed at 4°C per hour, or cooled at 0.5°C per hour and warmed at 10°C per hour. The optimal cooling and warming rates are higher than for the study by Jacobsen *et al.* (1984) as only tissue slices were used, not the whole organs. Since the tissue was less bulky, the problems associated with dense cell packing would be diminished. Warming and freezing rates are therefore not independent, but interact, and the rate of warming appears to play a more complex role than previously thought. As Rowe (1967) observed, there has been little study devoted to this aspect of the freeze-thaw cycle, and it may well be of more importance than is usually attributed to it.

Although these studies have all concerned mammalian systems, it would be expected that optimal cooling and warming rates to be low for plant organs as well, for the reasons outlined above for animal organs. This theory has important implications for the results of the present experiments: it is possible that no plants survived the freezing stress not because the temperatures themselves were lethal, but because the rates of cooling and warming were too rapid for organ survival. As described above, the tillers were cooled at a rate of 6°C per hour, which is within the rates recommended in the literature to cause extracellular freezing. The exact rate of warming was not determined, but the tissue was quickly moved, after freezing, to a refrigerator set at 4°C. The warming rate was therefore probably quite rapid, certainly greater than the 1°C/min recommended by Jacobsen *et al.* (1984). Further experimentation is necessary to determine if the lethal stress was the temperatures or the rates used.

Intracellular freezing, because it occurs too rapidly to allow for water movement out of the cell, results in the formation of potentially damaging intracellular ice (Burke *et al.* 1976, Levitt 1980, Quinn 1985). This type of freezing is always lethal if ice crystals are large enough to be detected microscopically (Burke *et al.* 1976). Some researchers (e.g. Mazur 1977, Sakai and Yoshida 1967) conclude that it is possible for plant cells to survive intracellular freezing, provided that both freezing and thawing rates are rapid enough to form very fine intracellular crystals not harmful to the cell, and then melt these fine crystals before they can grow to a dangerous size. However, Asahina (1978) found that such innocuous crystals could occur only at extremely rapid cooling rates, i.e. 1000°C per minute. Thus he concludes no plant cell in nature could survive intracellular freezing. Although the ice crystals are small they have higher surface energies than do larger crystals, and therefore agglomerate if the warming rate is slow enough. Tissues that have frozen intracellularly can only survive if warmed rapidly (Mazur 1977). Presumably the crystals damage the plasma membrane and organelle membranes, perhaps by piercing the membranes and destroying their semipermeability. The breakdown of cellular compartmentalization would release enzymes, leading to further damage of the injured tissue (Burke *et al.* 1976). Little is known about the processes and results of intracellular freezing, and thus there is some controversy about what happens during this process.

During an extracellular freeze-thaw cycle such as that described above, cells will be subjected to several types of stress: thermal, mechanical, chemical, and possibly electrical perturbations, all of which may interact resulting in injury (Kendall *et al.* 1985). Predominate among the injuries suffered are a loss of osmotic responsiveness and increased leakage of cytoplasmic solutes, which indicate membrane damage (Steponkus 1984).

Most cryobiologists now agree that extracellular ice does not cause damage by direct mechanical effects, but rather by a combination of low temperature effects, concentration of solutes, and most importantly, dehydration (Siminovitch and Clouthier 1983). Dehydration produces several physical changes: concentration of solutes, reduction in cellular water content, plasmolysis, pH changes, and changes in spatial distribution of macromolecules. In the past, there have been attempts to attribute all forms of injury to a single stress; it is more reasonable to assume that injury is the result of several stresses, since as mentioned above, during a freeze-thaw cycle, cells are subject to a multitude of stresses (Steponkus 1984). Several recent studies have concluded that changes in the protoplasmic membrane during freezing are a primary cause of injury (DeYoe and Brown 1979, Kendall *et al.* 1985, Quinn 1985, Santarius 1973, and Singh and Miller 1982). This theory proposes that cell survival is related to the integrity and fluidity of plasma membranes, and that the ultrastructure of the membrane bilayer is irreversibly altered when frozen (DeYoe and Brown 1979, Singh and Miller 1982). Evidence for this is given by the common symptoms of freeze-damaged plants: loss of osmotic responsiveness and increased efflux of cytoplasmic solutes (Steponkus 1984).

Plasma membranes are composed of a heterogenous lipid matrix arranged in a bilayer, which supports membrane proteins. These proteins are of two types: peripheral, which are attached to the membrane only by polar interaction, and integral which interact with the hydrocarbon domain of the matrix (Quinn 1985). The membrane lipids are amphipathic and can form two types of structures in water: bilayers or hexagonal-II structures , as well as intermediates of these two (Fig. III-6). Reduction in temperature initiates a physical change of state in the hydrophobic lipid matrix, and results in a crystalline or solid phase, which increases the susceptibility of the membrane to stress (DeYoe and Brown 1979). As the membrane is further cooled, lipids which tend to form non-bilayer structures or have high gel-phase melting temperatures (the gel-phase melting or transition temperature is the highest temperature at which lipids are present in the gel, not the crystalline phase) become phase separated into domains of pure gel-phase lipids (Quinn 1985). Where these phase separations occur, changes aren't likely to be reversed upon reheating (Kendall *et al.* 1985). In theory, damage occurs upon reheating, when these phase-separated lipids would form non-bilayer structures e.g. inverted micelles (Fig. III-7) thereby destroying membrane integrity (Quinn 1985). In the plasma membranes of winter wheat, freezing induced a dramatic increase in the highest temperature at which gel-phase lipids remained (Kendall *et al.* 1985). The freeze-stressed membranes also had accumulations of free fatty acids, the product of phospholipid degradation, which could accumulate in lipid bilayer and thus contribute to the formation of gel-phase domains. Membranes with substantial proportions of these gel-phase domains were found to be readily permeable to solutes and ions (Kendall *et al.*

Page 88 has been removed because of the unavailability of copyright permission. Page 88 contained information on molecular arrangements in structures, in mixtures of membrane lipids (from Quinn, 1985).

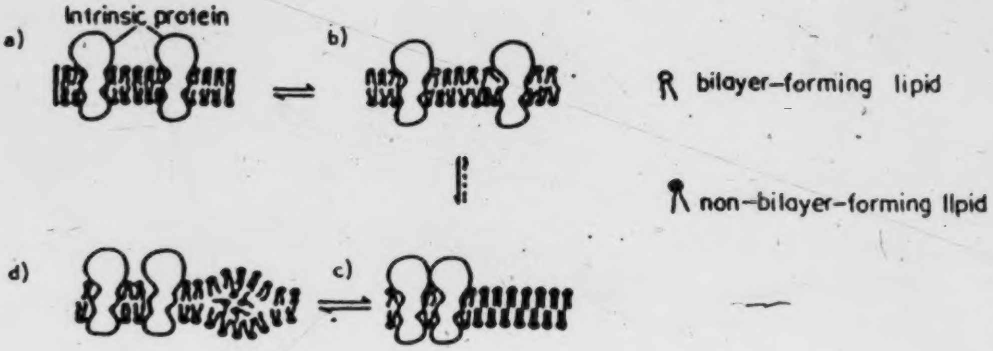

Intrinsic protein
a)
b)
c)
d)
bilayer-forming lipid
non-bilayer-forming lipid

Fig. III-7. Schematic representation of the phase separations in biological membranes associated with cooling membranes from their growth temperature (a) to a temperature below the gel to liquid-crystalline phase-transition temperature (c) and reheating to the growth temperature (d). Lipids that tend to form hexagonal-II phase are indicated by a solid head group. See text for explanation of events. Adapted from Quinn 1985.

1985) The results of Singh and Miller (1982) also support this model; they studied freezing in cold-hardened and nonhardened winter rye and found that in nonhardened cells as water is removed from the cell during freezing, the planar bilayer phospholipids would reorganize irreversibly into a less ordered amorphous state, whereas phospholipids of hardened cells could retain their ordered lamellar lattice. Also, in nonhardened tissue during freezing, there is irreversible fusion ("roll-up") of cellular membranes to form densely packed multibilayered vesicles. If either of these changes occurs in a cell during freezing, it may begin to expand upon thawing (rehydration), but it will lyse before reaching its original size (Singh and Miller 1982). Harvey *et al* (1982) examined two groups of cool climate plant species: woody perennials and cereals, and compared the phase separation temperature to the temperature at which intact plants were damaged. The phase separation temperatures of the two groups are similar, but the frost sensitivity is very different. The woody species tolerate temperatures well below their phase separation temperature, while the cereals are damaged about 25°C above their phase separation temperatures. While this experiment indicates that phase separation itself is not the lethal stress in freezing, the correlation between the temperatures of phase separation and frost sensitivity suggests that lipid properties influence freezing tolerance in cereals. Quinn's (1985) model also predicts that some species possess homeostatic mechanisms that preserve properties of membrane lipid matrix. There is evidence for such biological reactions that sense the physical changes in membranes subjected to temperature changes. DeYoe and Brown (1979) found that chloroplast membranes of white pine maintain lamellar viscosity by increasing lipid unsaturation, and are able to tolerate freezing desiccation by increasing the water-binding capacity of the lamellae.

Since frost tolerance is at its lowest during the summer, all plants in these experiments were unhardened, thus they would not have any of these protective mechanisms conferred by hardening. Therefore, when subjected to sub-zero temperatures during the growing season, the only means of surviving is avoidance of freezing by deep supercooling and waiting out the brief cold spell. The plants would have some protection from cryoprotectants; plants have the greatest variety of cryoprotective devices, as they have limited options for avoiding freezing (Meryman *et al.* 1977). One such device is increased synthesis of intracellular solutes, usually sugars, that increase the osmolality of the cell; meaning that the extracellular osmolality must be higher to achieve the same dehydration of the cell. Plants growing in favorable environments would tend to produce more carbohydrates, incidentally giving them a greater degree of cryoprotection. The lower freezing point of plants grown in the warm regime is probably due to an increased level of cryoprotectants such as sugars and organic acids, that would be more abundant under this regime. *P. alpina* from 2000 metres and *P. pratensis* did not grow well under the cool temperature regime, so probably had lower synthesis of carbohydrates and therefore froze at warmer temperatures than the other grasses. The results of Santarius' (1973) study indicate that in the absence of cryoprotectant agents, freezing stress would lead to irreversible inactivation of electron transport and photophosphorylation; the addition of sugars to the membrane prevented this inactivation. The protective effect is a function of both concentration and molecular weight of the sugars (Santarius 1973). There are two mechanisms by which sugars are able to protect membranes during freezing. Firstly, such non-toxic solutes serve to dilute the concentration of toxic solutes during freezing and secondly, the OH group of the sugars has a high affinity for water and thus can influence water binding (Santarius 1973). For example, they can bind to proteins and thus prevent their denaturation (Alexandrov *et al.* 1970). Quinn (1985) states that cryoprotectants are able to alter the phase-transition properties of membrane lipids so as to prevent the formation of separate gel-phase domains. This alteration is due to their binding of water moleclues, which causes a reduction in the crystalline-gel phase transition temperature.

3.3.3 Differences in Resistance between Tissues

For these grass species, roots were found to freeze earliest, followed by the culm and lastly by the leaves. These results agree with those of other studies that roots are the organs most sensitive to cold and leaves tend to be most resistant (Larcher 1973). This pattern is partly due to water content: roots contain more water and so are killed at warmer temperatures, since at higher moisture contents most of the water freezes rapidly at a single freezing point resulting in intracellular freezing (Burke *et al.* 1976). Another reason for this pattern is that the prevailing temperature under natural conditions will affect freezing resistance; the above ground parts have greater resistance than those below ground (Levitt 1980). In nature, roots and crowns are able to survive most freezing conditions because the regenerative growing points are at or below the soil surface and thus are protected by residual soil heat and insulating snow cover (Burke *et al.* 1976). As well, there would be water movement from the warmer roots towards the colder shoots. This is demonstrated by the equation for Gibbs free energy (G),: $G = E + PV - TS$ where E = internal energy, PV = pressure-volume product, T = absolute temperature, and S = entropy. Increasing the temperature increases the internal energy, and consequently, the free energy or water potential. Thus water will move from points of high to low temperature (Salisbury and Ross 1978). Gerber and Hashemi (1965) found that for nine citrus fruit species, the freezing points of detached leaves were within 2°C of the killing temperature of intact plants. In constrast, in these experiments, detached leaves had a significantly lower freezing temperature than did attached leaves. It is possible that the detached leaves could have become dessicated while slowly being cooled in the freezer.

3.3.4 Heat Tolerance

Of the three species, *T. spicatum* is most heat sensitive and *P. pratensis* is the least heat sensitive, based on percent surviving at 45°C. The fact that all plants were killed when exposed to 50°C is not surprising as this temperature is often cited as the lethal temperature for many organisms.

3.3.5 Heat Injury

This area has not been as extensively studied as has low temperature injury perhaps because heat injury is not as complex. Heat causes death by damaging membranes (Santarius 1973) and by denaturation of proteins, especially enzymes (Larcher 1973, Alexandrov *et al.* 1970). This damage inhibits the plant's metabolism resulting in decreased productivity or even starvation if the photosynthetic enzymes are affected, as well as accumulation of toxic decomposition products, if protein metabolism becomes disorganized. One of the primary causes of heat injury is uncoupling of phosphorylation from electron transport (Santarius 1973). Santarius (1973) found that high-temperature inactivation of electron transport in chloroplast membranes suspended in water occurs within two to three minutes at 45°C. Thus, as in frost sensitivity, membranes are a key factor in heat sensitivity. Sugars also serve as cryoprotectants of membranes during high temperature stress (Santarius 1973). Of the two protective mechanisms Santarius identified for cryoprotectants, the second mechanism (binding of OH groups to water) would also stabilize membranes against heat stress. The rise in heat resistance in winter occurs passively as a result of biochemical changes that confer frost resistance (Santarius 1973). The rise in heat resistance during summer is due to a different mechanism, perhaps the result of changes in membrane proteins (Alexandrov *et al.* 1970, Levitt 1980).

3.4 Conclusions

The three grass species studied have similar levels of frost avoidance, but *T. spicatum* has slightly greater frost avoidance which would give this species an advantage in dealing with the periodic frosts which occur during the growing season in the alpine.

For all three species, the freezing temperatures were fatal; damage was probably due to irreversible physical changes in the plasma membranes, and effects of dehydration; it is possible that the freezing rates were too rapid for organ survival.

Of the three grasses, *T. spicatum* is the most heat-sensitive and *P. pratensis* is the least heat-sensitive. All three species were killed by exposure to 50°C. The range of high temperatures used in this experiment were similar to those observed on several alpine disturbed sites; both air and soil temperatures can be very high, even lethal, in such open disturbed sites (Ballard 1972). These temperatures have important implications for the distribution and development of plants, particularly where the climate is marginal for the development of major plant cover types (Ballard 1972), as is certainly the case on such spoils.

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4. Carbon Dioxide Gas Exchange

4.1 Introduction

In alpine regions, plant productivity is primarily limited by the short growing season and low temperatures (Billings *et al*. 1966). Plants growing in alpine environments must produce enough assimilates during the short cold growing season to survive a long winter and to provide the substances needed for rapid growth at the start of the next growing season (Billings *et al*. 1966, Moore *et al*. 1973). Alpine plant species possess several adaptations allowing them to maximize their carbon gain during this brief cool growing season; most important among these adaptations are (1) the rapid development of photosynthetic tissue (Caldwell *et al*. 1978), (2) the capacity for photosynthesis over a wide temperature range. Several studies have suggested that alpine plants in general possess higher temperature optima for photosynthesis than arctic plants (Mooney and Billings 1961, Billings *et al*. 1971, Moser *et al*. 1977).

Alpine temperature regimes are also characterized by temperature fluctuations over a wide range during each daily cycle, and considerable fluctuations are possible within relatively short intervals during the day or night. Maintenance of a positive carbon balance over this range of environmental conditions would require the ability for a photosynthetic apparatus that could rapidly adapt to changing conditions. This reaction has been termed photosynthetic acclimation potential, and is defined as "...the ability of a given genotype to change its photosynthetic characteristics in an adaptive manner in response to changes in environmental conditions such as light, temperature, or water regime." (Mooney *et al*. 1978a). Different species, and populations within species, may possess different acclimation potentials. This has important implications for reclamation purposes, as plants which can achieve high photosynthetic rates over a wide temperature range may well be more useful in the field than one with a high rate of photosynthesis at only one temperature range (Ku and Hunt 1973, Musselman *et al*.

1975).

For alpine revegetation, it might be expected that the most successful plants would be those which possess: (1) high photosynthetic activity at low temperatures, and (2) a high acclimation potential, i.e. high photosynthetic rates over a relatively wide temperature range. The first hypothesis was tested by determining the temperature response of the carbon dioxide gas exchange process in the two native grass species, *Poa alpina* and *Trisetum spicatum*, and one introduced species, *Poa pratensis* var. Nugget. The second hypothesis (2) was tested by growing the grass species under two different growth regimes to determine the species' relative abilities to maintain their photosynthetic rates over a wide temperature range. It might be expected that indigenous alpine grasses will be more successful for reclamation of high elevation disturbances than introduced species, given that in any environment, the most successful plant populations are those that have evolved the most appropriate physiological mechanisms. The native species have the advantage of already possessing the necessary adaptations to the alpine environment.

The final section of this study examines three populations of *Poa alpina* to determine if there is any differentiation of photosynthetic characteristics among them. *P. alpina* is a widely distributed species successful in a range of habitats, and is an early invader of disturbed areas. This success could be due to a broad ecological amplitude (i.e. high acclimation potential) among members of the species or due to the development of populations that have become adapted to their local environment --i.e ecotypes, as described in the General Introduction. Several studies have indicated that the photosynthetic apparatus is very sensitive to environmental variation and tends to adjust to the specific environment such that productivity is maximized (Bjorkman and Holmgren 1963). Treharne and Eagles (1970) concluded that climatic races have been shown to differ markedly in the rates of photosynthesis and respiration, and the difference can often be explained in terms of physiological adaptation to their local environment. Conversely, Williams and Kemp (1976) report that many photosynthesis studies indicate limited formation of ecotypes that were photosynthetically temperature-sensitive. Thus the third objective of this study was to answer the question "what is the relative effect of phenotypic temperature acclimation on metabolic rates *versus* the effect conferred by the agamotype?" in populations of *Poa alpina* from different habitats.

4.2 Materials and Methods

4.2.1 Plant Material

All plants of *Poa alpina* used in these experiments were provided by the Vegetation Group at the Alberta Environmental Centre; a description of the origin and collection of these plants is given in the General Introduction. Plants of *Trisetum spicatum* and *Poa pratensis* were grown from seed in controlled environment chambers at the University of Alberta. Ten plants of each species (for *P. alpina*, ten of each population) were divided between a warm regime chamber and a cool regime chamber as described in the General Introduction. Plants were not used in the experiments until they had been growing in the growth chambers for about two months.

4.2.2 Gas Exchange Equipment and Operation

Carbon dioxide gas exchange was measured for whole grass tufts using the building's air supply in an open system (see Sestak *et al* 1971) with a Beckman Model 865 infrared gas analyzer (IRGA) (Fig. IV-1). The IRGA was adjusted for a sensitivity of 0 to 100 ppm carbon dioxide differential. Output of the IRGA together with various temperatures was recorded on a six channel recorder. Ambient air was drawn from three metres above the chambers, and was mixed continuously by a fan to minimize layering and fluctuations in carbon dioxide concentration. A sample of this air was

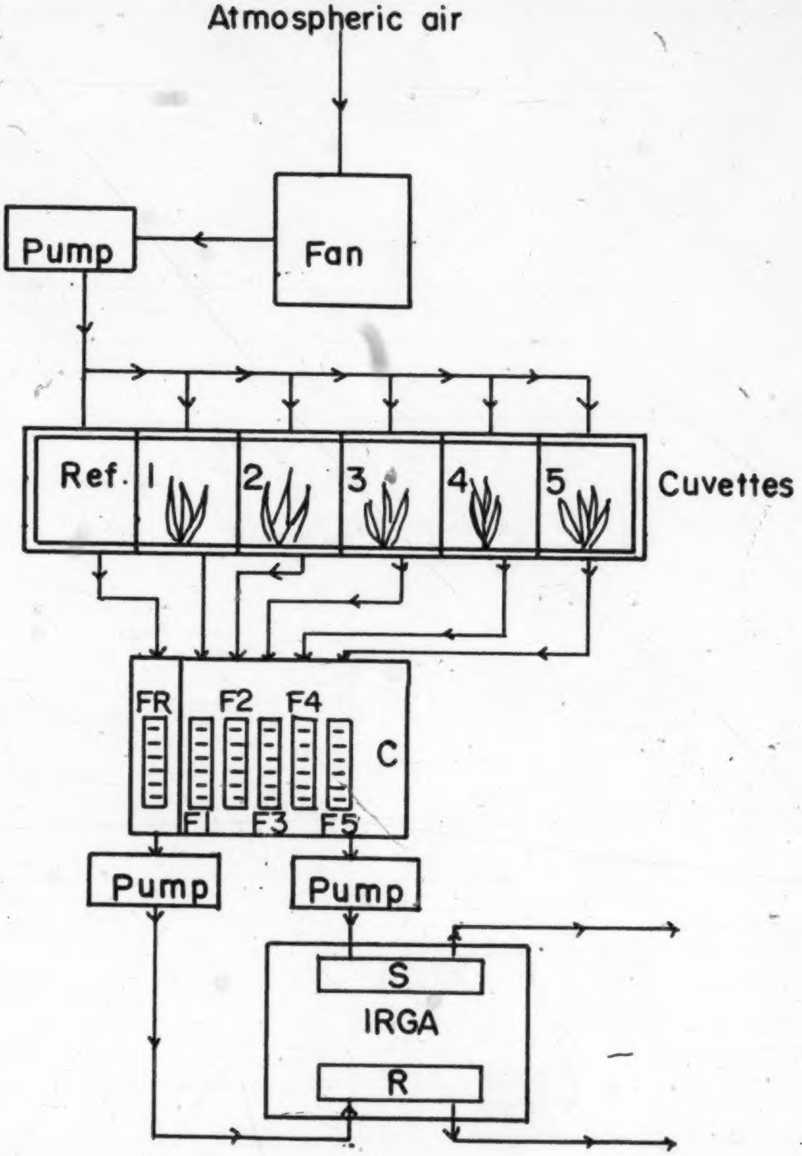

Atmospheric air
Fan
Pump
Ref.
1
2
3
4
5
Cuvettes
FR
F2
F4
C
F1
F3
F5
Pump
Pump
S
IRGA
R

Fig. IV-1 Diagram of open gas-exchange system. C = channel selector; F_n = flow meters; S = sample cell of IRGA; R = reference cell of IRGA. Arrows indicate direction of air flow.

passed through a dessicating column of silica crystals and and from there to the reference cell of the IRGA, passing through a container to compensate for the volume of the cuvette. The remainder of the air was pumped to the cuvettes at a uniform flow rate of 60 liters per hour per cuvette. Air entered the chambers under force to ensure mixing. The system allowed five replicates to be run during each experiment. The cuvettes were made of Plexiglass, with a volume of .8 litres; the cuvettes were mounted in a water tank with a volume of 116.5 litres. Air was automatically drawn from each cuvette passed through a column of dessicant and then routed through the sample cell of the IRGA by means of an ADC six-channel selector. The selector was set on a one-minute dwell time for each chamber, followed by a one minute purge of the sample chamber, before the next cuvette was sampled in sequence. Temperature in the cuvette could be varied from 5° to 40° C, and was precisely regulated (0.25°C) by circulating water from either a heating unit or a refrigeration unit through the water tank. Temperatures of water, air, soil, and leaves were monitored using copper-constantan thermocouples. Irradiance of the plants was provided by one sodium and one mercury light suspended 75 cm above the cuvettes. These lamps supplied 750 - 850 micromol/ m^2/ second at the cuvette level. The system was allowed about one hour to equilibrate under each condition before any measurements were taken. The IRGA was calibrated regularly (usually once/week) with standard gases of known carbon dioxide concentrations (325 ppm and 395 ppm) to give a calibration factor used in the calculation of gas exchange (see below). Mean exchange values were calculated for each experimental condition; the standard error of the mean was calculated. At the completion of an experiment the above ground growth was clipped, oven-dried at 80°C for 48 hours, and weighed.

4.2.3 Gas Exchange Procedure

Ten to twelve hours before plants were required for carbon dioxide gas exchange measurements, the roots and soil were sealed off from the shoots by a double layer of aluminum foil; spaces around the grass culms were sealed with Vaseline. The plants were then fitted into the cuvettes, and the lids were placed on top of the cuvettes, sealed with Vaseline and clamped firmly into place. The pots remained outside, at ambient air temperatures. Measurements were initially conducted at leaf temperatures of about 20°C; i.e. ambient temperature. After steady-state photosynthesis rates were achieved, the temperature was increased in 2° - 5° C increments, by increasing the temperature of the heating unit,. The maximum leaf temperature reached using the heater was 35° C; after steady-state levels were recorded at this temperature, the heater was turned off and the refrigeration unit turned on, which lowered the temperature very gradually (about 2° C/ hour). Therefore the plants were not stressed by the change in temperature. The lowest leaf temperature possible with the lamps on was 5°C. To obtain lower temperatures, special experiments were done, which are described later. Respiration response to temperature followed the same general procedure as photosynthesis measurements; lamps were shut off, and the cuvettes were covered with black cloth for thirty minutes before measurements were made.

The amount of carbon dioxide released or taken up by the plant was determined from the difference between the carbon dioxide concentration of the cuvette air and ambient air; this difference (d) was recorded on chart paper using a Hewlett-Packard chart recorder. Rates of respiration and photosynthesis were calculated using the formula:

$$\text{mg } CO_2/\text{g DW/h} = (d)(.00184 \text{ mg } CO_2)(60 \text{ l/h})(\text{calibration factor})/\text{Dry Weight of plant.}$$

(.00184 = mg CO_2 in 1 litre CO_2 at 20°C, for lower temperatures, different values were used; 60 l/h is the flow rate used).

The foil and Vaseline were removed immediately after the experiment was completed and the leaves were harvested; this treatment did not appear to have any adverse effects on plant growth, as all plants thus treated had new, healthy growth within a few days after the experiment.

4.2.4 Low Temperature Measurements

The grasses were still photosynthesizing actively at 5°C and thus in order to obtain the lower temperature compensation point for photosynthesis, the grasses had to be cooled below 0°C. Several techniques were attempted to achieve such cold temperatures; the most successful involved the use of ice and salt. The plants were fitted into the cuvettes as before, but instead of filling the tank with water, it was filled with thin, alternating layers of coarse salt and ice. The sodium lamps were not turned on for 20 - 30 minutes, to allow the plants to cool below zero. Leaf, air, soil temperature and carbon dioxide concentration were continuously measured, so that the compensation point would be recorded, and also to monitor for any "reaction respiration" (Bauer *et al.* 1975) that would indicate that the plants were under stress. The ice-salt mixture caused the plant temperature to decrease gradually from 2°to -10°C; -10° seems to be the coldest possible temperature using this method, with the lights on. When the leaf temperature reached 0°C again, the system was flushed with water, and the plant temperature allowed to gradually increase to about 20°C (ambient temperature). Readings were taken at regular intervals of 2°- 5°C at this time, to determine whether the exposure to subzero temperatures had inhibited photosynthesis rates. Plants were not clipped immediately following gas exchange measurements, but were observed over the next few days to monitor low-temperature damage. Photosynthesis was re-measured within twenty-four hours to determine if there had been any long-term inhibition of photosynthesis.

4.3 Results

A comparison of the effects of growth temperature on net photosynthesis and dark respiration rates in the three species (Fig. IV-2 to IV-6; based on Tables IV-2 and IV-3) indicates that: (1) The native species had broad temperature response curves for net photosynthesis and were active from a few degrees above 0°C to 20°C, whereas *P. pratensis* had a very narrow temperature reponse curve and was most active only from 10°to 20° C; (2) temperatures greater than 20° - 25 ° C caused a substantial decrease in net photosynthesis in *P. alpina* and *T. spicatum*; net photosynthesis in *P. pratensis* was not significantly affected by these temperatures; (3) *P. pratensis* had much higher rates of net photosynthesis than either native species (11.5 mg CO_2 /g DW/h *vs.* 5 - 6 mg CO_2 /g DW/h). (4) the dark respiration rates of the native species tended to be higher when grown in the cool regime, whereas the dark respiration rate of *P. pratensis* was higher when grown at warm temperatures; (5) in all species the optimum temperature for photosynthesis shifted upwards when the plants were grown under the warm temperature regime; (6) in *P. alpina* from all three elevations the net photosynthesis rates decreased at the warm growth temperatures; this decrease also occurred in *P. pratensis* to a lesser extent.

A one-way ANOVA of the net photosynthetic rate at the optimum temperature for photosynthesis in each species was done, and indicated that there was a significant difference among the species (Table IV-1). Figure IV-7, based on the data in Tables IV-1, IV-2 and IV-3, summarizes the major results of the photosynthetic temperature response in the three grass species. The most important points are: (1) Under both cool and warm growing temperatures, the indigenous species had lower optimum temperatures for photosynthesis than does *Poa pratensis*.

(2) There was no significant difference between any population of *P. alpina* and *Trisetum spicatum* with respect to the rate of carbon dioxide fixed or the optimum photosynthetic temperature.

Fig. IV-2. Effects of leaf temperature on net photosynthesis at 750 - 850 micromol/m^2/sec., and dark respiration of *Poa alpina* plants (from 2700 m), grown under two temperature regimes.

Values are the mean of ten replicates.

Verticle bars represent standard error of the mean.

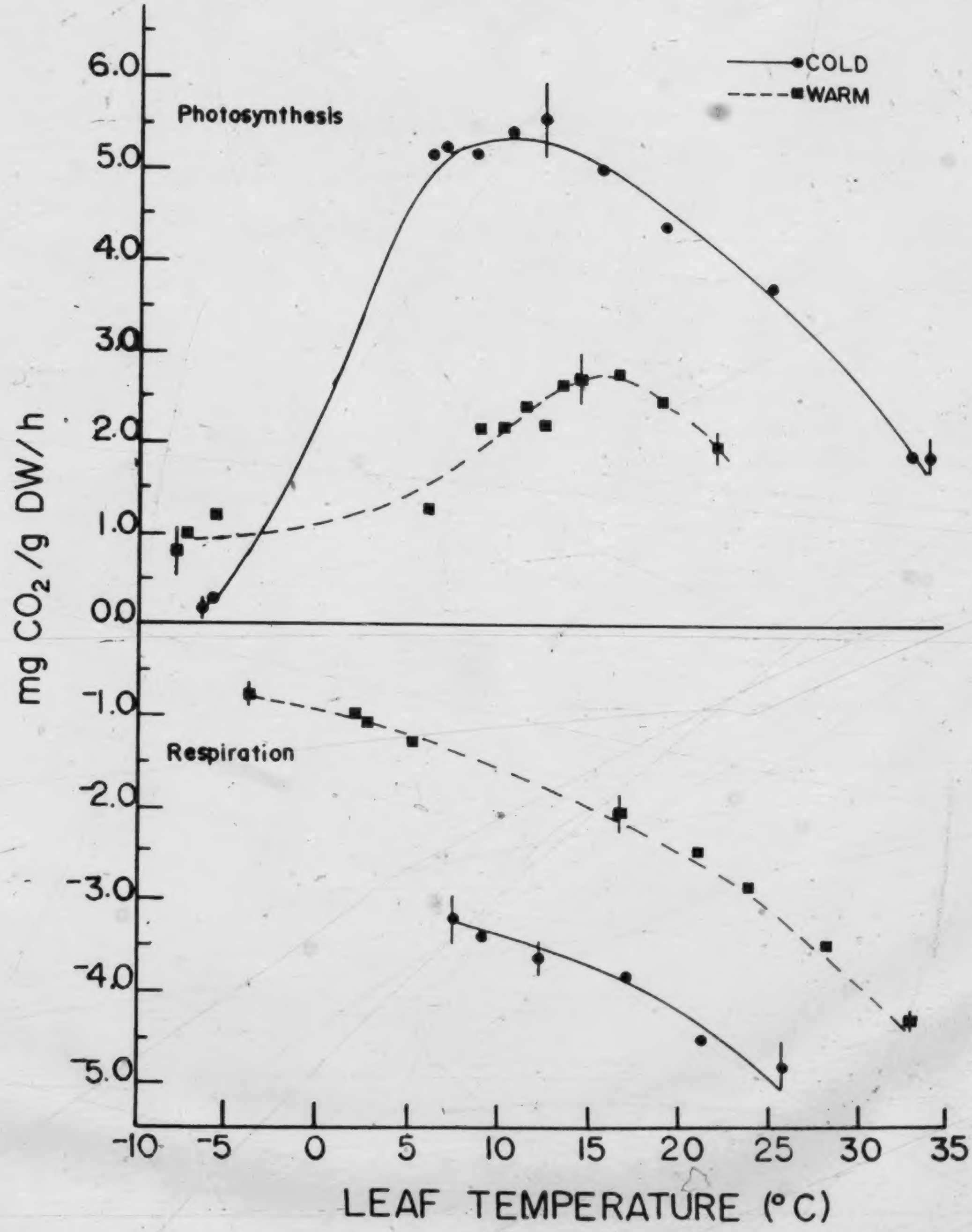

COLD
WARM
Photosynthesis
Respiration
6.0
5.0
4.0
3.0
2.0
1.0
0.0
-1.0
-2.0
-3.0
-4.0
-5.0
mg CO_2 /g DW/h
-10
-5
0
5
10
15
20
25
30
35
LEAF TEMPERATURE (°C)

Fig. IV-3. Effects of leaf temperature on net photosynthesis at 750 - 850 micromol/m^2/sec., and dark respiration of *Poa alpina* plants (from 2300 m) grown under two temperature regimes.

Values are the mean of ten replicates.

Verticle bars represent standard error of the mean.

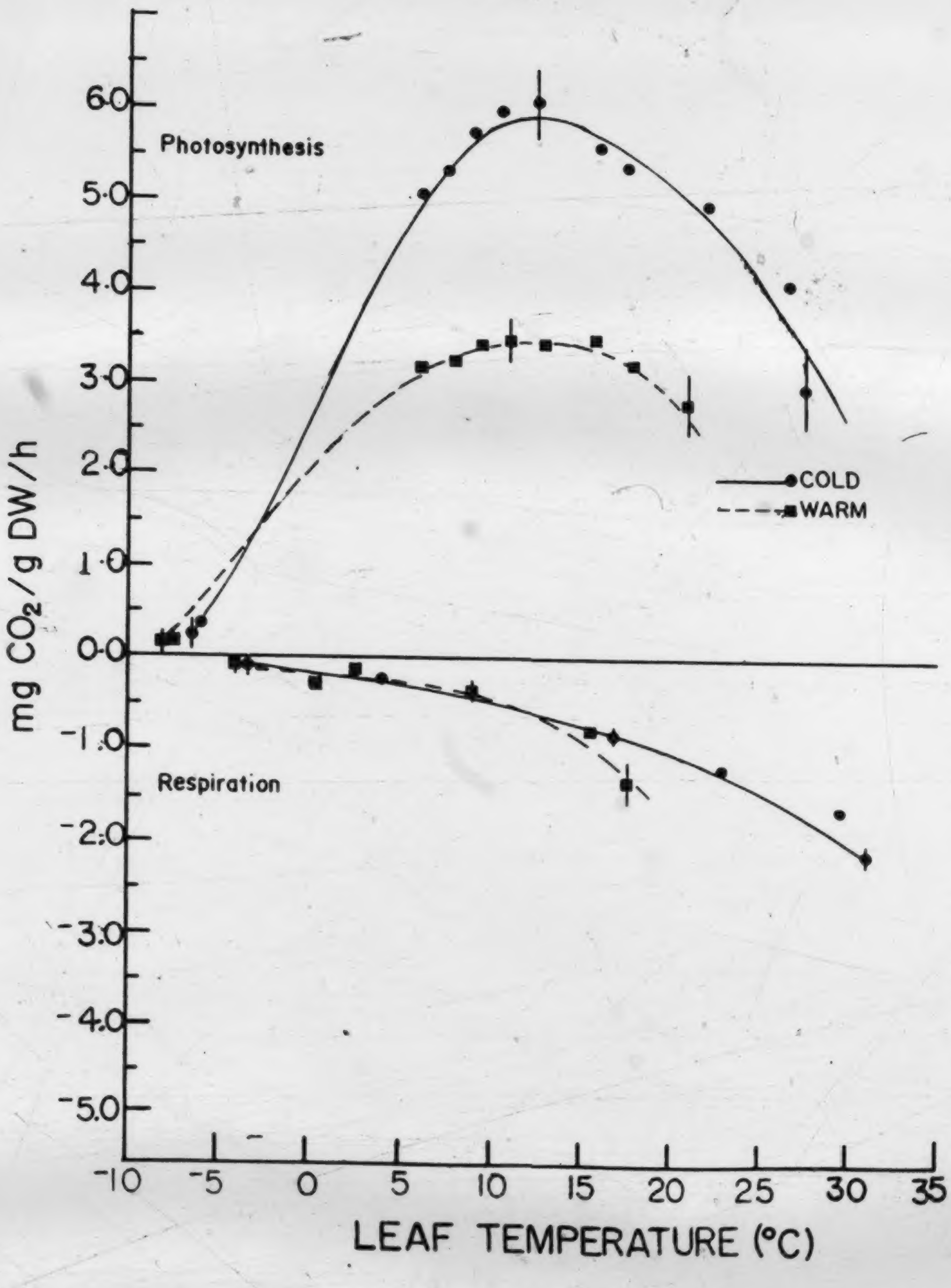

6.0
5.0
4.0
3.0
2.0
1.0
0.0
-1.0
-2.0
-3.0
-4.0
-5.0
mg CO$_2$/g DW/h
Photosynthesis
Respiration
COLD
WARM
-10
-5
0
5
10
15
20
25
30
35
LEAF TEMPERATURE (°C)

Fig. IV-4. Effects of leaf temperature on net photosynthesis at 750 - 850 micromol/m^2/sec., and dark respiration of *Poa alpina* plants (from 2000 m), grown under two temperature regimes.

Values are the mean of ten replicates.

Verticle bars represent standard error of the mean.

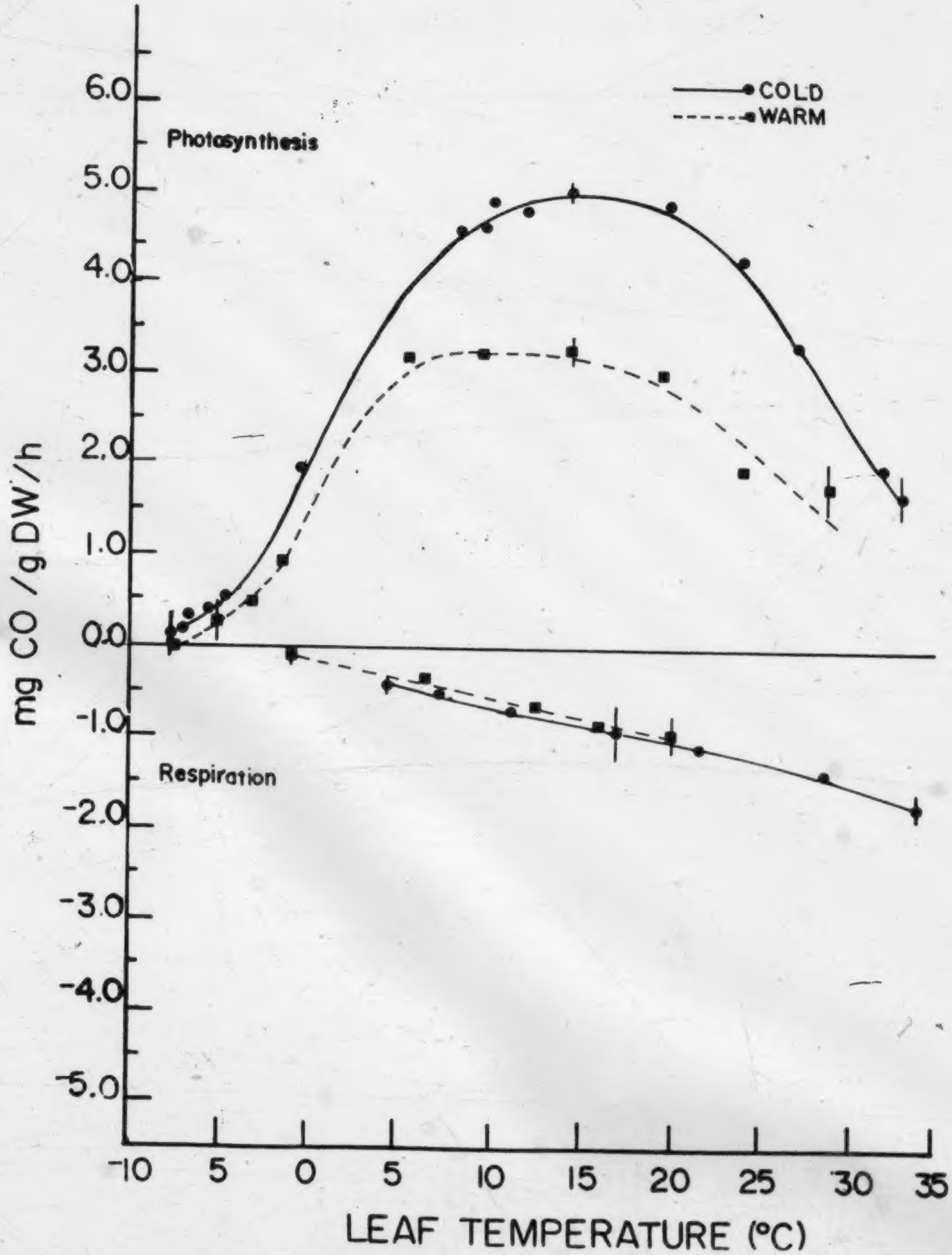

COLD
WARM
Photosynthesis
Respiration
mg CO /g DW/h
6.0
5.0
4.0
3.0
2.0
1.0
0.0
-1.0
-2.0
-3.0
-4.0
-5.0
-10
5
0
5
10
15
20
25
30
35
LEAF TEMPERATURE (°C)

Fig. IV-5. Effects of leaf temperature on net photosynthesis at 750 - 850 micromol/m^2/sec., and dark respiration of *Trisetum spicatum* plants, grown at two temperature regimes. Values are the mean of ten replicates. Verticle bars represent stand error of the mean.

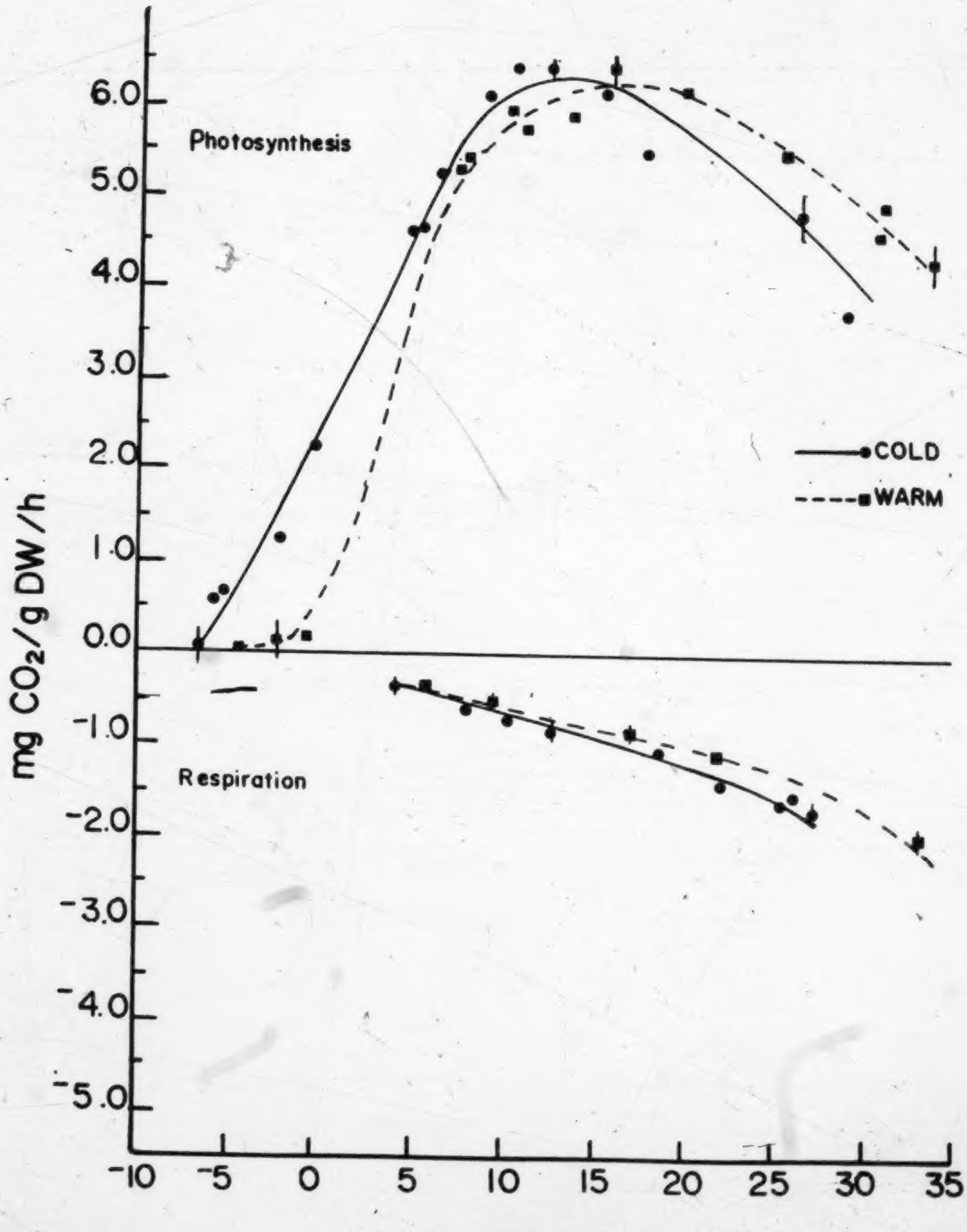

mg CO$_2$/g DW/h
6.0
5.0
4.0
3.0
2.0
1.0
0.0
-1.0
-2.0
-3.0
-4.0
-5.0
Photosynthesis
Respiration
COLD
WARM
-10
-5
0
5
10
15
20
25
30
35
LEAF TEMPERATURE (°C)

Fig. IV-6. Effects of leaf temperature on net photosynthesis at 750 - 850 micromol/m^2/sec., and dark respiration of *Poa pratensis* plants, grown at two temperature regimes. Values are the mean of ten replicates. Vertical bars represent standard error of the mean.

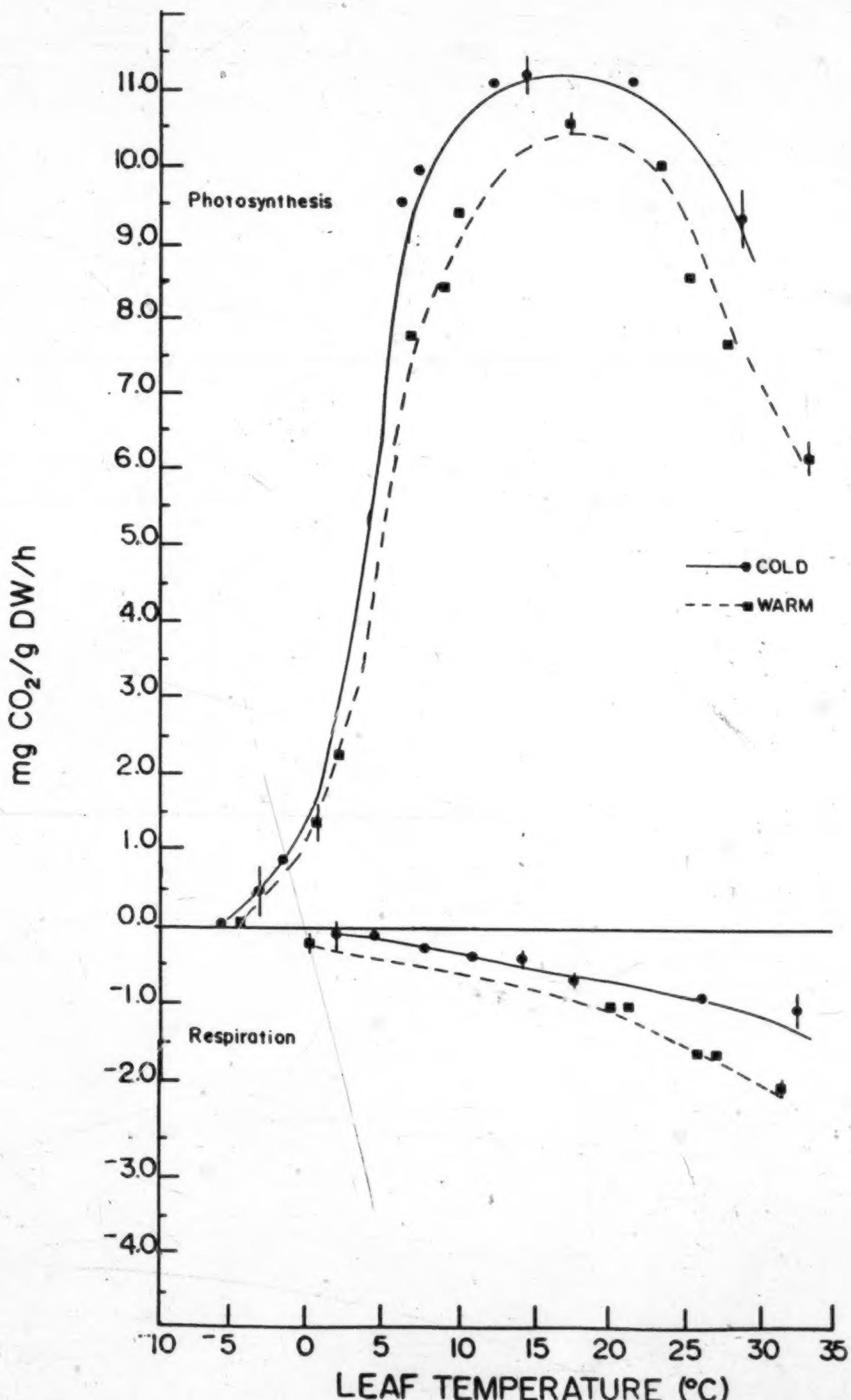

Photosynthesis
Respiration
mg CO$_2$/g DW/h
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
-1.0
-2.0
-3.0
-4.0
COLD
WARM
-10
-5
0
5
10
15
20
25
30
35
LEAF TEMPERATURE (°C)

Table IV-2. Photosynthetic rate vs. leaf temperature. Rates expressed in absolute values (mgCO$_2$/g DW/h) and as % of maximum rate. Plants grown under cool regime of 10°C day/5°night. Values are the mean of 10 replicates; standard error of the mean is given for absolute rates.

Poa alpina 2700 m			*P. alpina* 2300 m			*P. alpina* 2000 m		
Temp. °C	Absolute rate	% of max.	Temp. °C	Absolute rate	% of max.	Temp. °C	Absolute rate	% of max.
-6.50	0.16±0.03	2.87	-6.50	0.22±0.06	3.62	-7.75	-.039±0.04	0.78
-6.0	0.25±0.04	4.49	-6.00	0.37±0.13	6.09	-6.50	.300±0.07	5.96
6.25	5.14±0.18	92.45	6.00	5.07±0.34	83.38			
7.00	5.23±0.15	94.06	7.25	5.22±0.35	85.85			
			7.50	5.33±0.35	87.66			
8.75	5.17±0.15	92.98				8.50	4.63±0.03	92.04
			9.00	5.76±0.59	94.74	9.00	4.65±0.01	92.44
10.50	5.42±0.17	97.48	10.00	5.96±0.38	98.02	10.00	4.96±0.01	98.61
12.50	5.56±0.37	100.00	12.50	6.08±0.40	100.00	12.00	4.84±0.02	96.22
						14.50	5.03±0.01	100.00
15.75	5.01±0.16	90.11	15.50	5.58±0.45	91.78			
			16.00	5.60±0.49	92.10	16.50	4.69±0.04	93.24
19.50	4.44±0.19	79.86	17.50	5.41±0.49	88.98			
						20.00	4.91±0.18	96.61
23.00	3.70±0.19	66.55	22.00	4.95±0.46	81.41			
			26.50	4.10±0.47	67.43	24.00	4.37±0.22	86.88
			27.50	2.99±0.43	49.17	27.25	3.38±0.27	67.20
						32.00	2.01±0.31	39.96
33.25	1.89±.20	33.99				33.00	1.66±0.24	33.00
34.00	1.65±.03	29.68						

Table IV-2. (Continued)

Poa pratensis			*Trisetum spicatum*		
Temp. °C	Absolute rate	% of max.	Temp. °C	Absolute rate	% of max.
			-6.75	0.01±0.09	0.16
-3.00	0.47±0.08	4.01			
			-5.50	0.68±0.22	10.58
-1.50	0.89±0.13	7.59			
			5.00	4.61±0.17	71.69
6.50	9.53±0.02	81.31	5.25	4.67±0.11	72.62
7.75	9.87±0.25	84.21			
9.25	9.69±0.23	82.68	9.25	6.12±0.79	95.18
			10.75	6.43±0.10	100.00
12.50	11.65±0.51	99.40	12.75	6.40±0.14	99.53
14.75	11.72±0.42	100.00			
			15.75	6.13±0.15	95.33
			17.50	6.22±0.11	96.73
22.00	11.66±0.38	99.49			
23.50	10.96±0.53	95.32	23.00	5.49±0.18	85.38
			26.50	4.85±0.18	76.42
27.25	11.02±0.26	94.02			
29.00	9.40±0.18	80.20	29.25	3.73±0.33	58.00
			31.25	4.55±0.25	70.76

Table IV-3. Photosynthetic rate vs. leaf temperature. Rates expressed in absolute values (mg CO_2/g DW/h) and as % maximum rate. Plants grown under warm regime of 25°C day/5°C night. Values are the mean of 10 replicates; standard error of the mean is given for absolute rates.

Poa alpina 2700 m			*P. alpina* 2300 m			*P. alpina* 2000 m		
Temp. °C	Absolute rate	% of max.	Temp. °C	Absolute rate	% of max.	Temp. °C	Absolute rate	% of max.
-8.00	0.80±0.25	28.99	-8.00	0.14±0.14	4.01	-7.50	0.20±0.06	0.61
-6.00	1.20±0.10	43.48	-6.00	0.16±0.04	4.58	-3.00	0.98±0.15	30.00
						-0.50	1.96±0.38	60.00
6.00	1.26±0.09	45.65	6.25	3.17±0.23	90.83	2.00	2.75±0.49	94.10
			8.00	3.25±0.26	93.12	5.50	3.19±0.49	97.55
9.00	2.16±0.12	78.26						
9.50	2.22±0.19	80.43	9.50	3.42±0.25	97.99	9.50	3.26±0.48	99.69
10.00	2.16±0.20	78.26						
10.50	2.16±0.21	78.26						
10.90	2.33±0.22	84.42	11.00	3.46±0.27	99.14			
11.50	2.39±0.21	86.59						
12.50	2.21±0.20	80.07						
13.00	2.17±0.19	78.62	13.00	3.45±0.29	98.85			
13.50	2.66±0.32	96.38						
14.75	2.70±0.30	97.83	14.25	3.42±0.24	97.99	14.50	3.27±0.42	100.00
16.75	2.76±0.29	100.00	15.85	3.49±0.24	100.00			
			17.75	3.21±0.24	91.98			
19.25	2.46±0.30	89.13	20.75	2.78±0.34	79.65	19.50	3.02±0.35	92.35
22.25	1.00±0.30	72.10				24.00	1.95±0.22	59.63
						29.00	1.80±0.18	55.04

Table IV-3. (Continued)

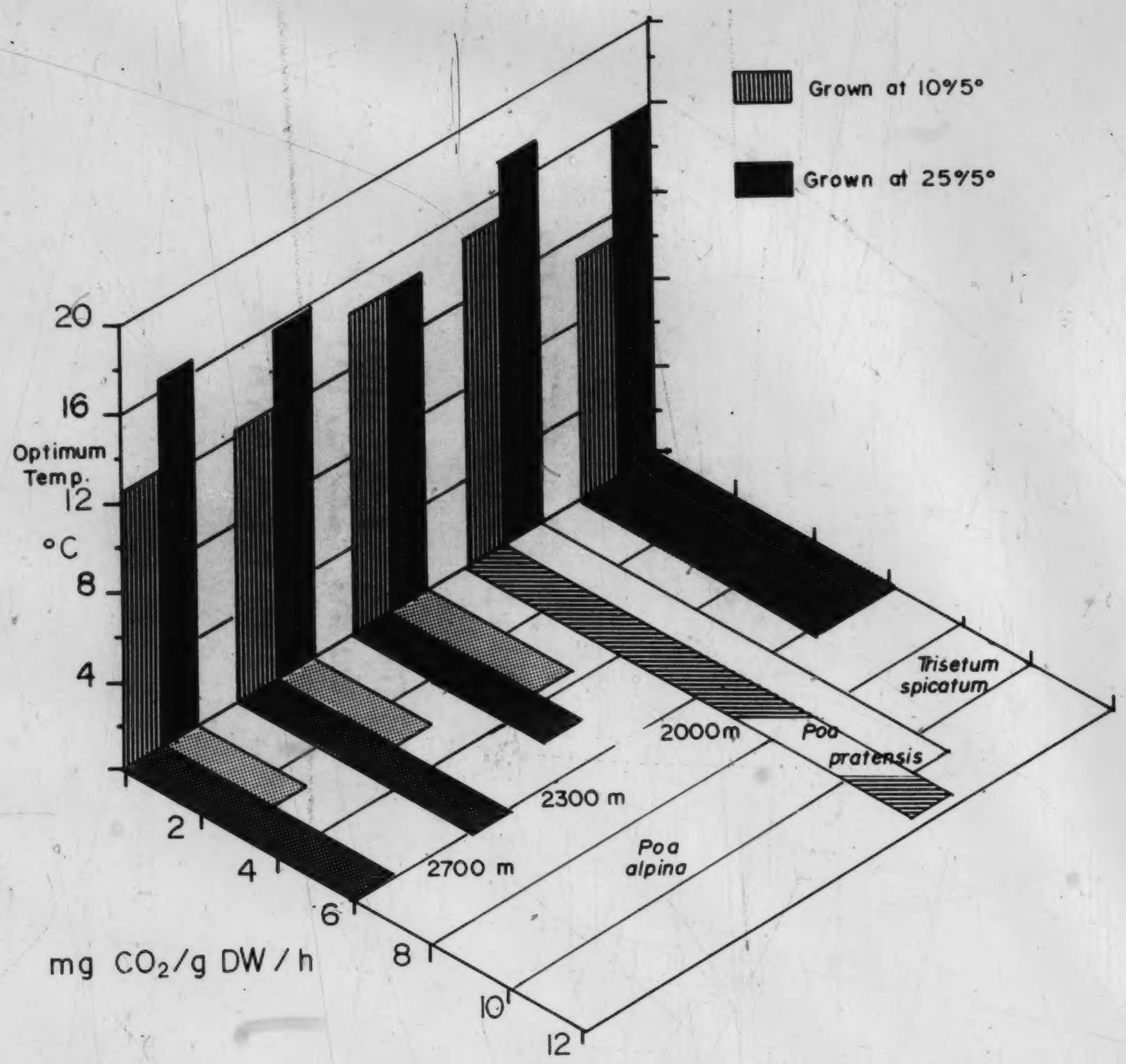
Poa pratensis			*Trisetum spicatum*		
Temp. °C	Absolute rate	% of max.	Temp. °C	Absolute rate	% of max.
-4.25	0.70±0.03	0.65	-4.25	0.07±0.03	1.09
2.25	2.29±0.19	21.36	-2.00	0.11±0.03	1.72
			6.50	5.29±0.17	82.66
7.00	7.80±0.16	72.76			
7.50	8.10±0.20	75.59	7.20	5.24±0.19	81.88
8.00	7.87±0.18	73.41	8.10	5.47±0.11	85.48
9.00	8.42±0.15	78.54	9.00	5.69±0.15	88.91
10.25	9.40±0.19	87.69	10.20	5.76±0.18	90.00
			11.80	5.76±0.12	90.00
			12.20	6.08±0.09	95.00
			13.80	5.91±0.10	92.34
15.50	9.68±0.20	90.30	15.90	6.40±0.10	100.00
17.50	10.72±0.22	100.00			
			20.30	6.17±0.14	96.41
23.25	9.54±0.29	88.99			
24.10	10.17±0.51	94.72	24.90	5.82±0.11	90.94
25.25	8.51±0.26	79.38			
			27.25	5.86±0.31	91.56
28.00	7.74±0.29	72.20			
30.00	7.17±0.26	66.88	31.50	4.91±0.29	76.72
			32.00	4.96±0.24	77.50
33.50	6.25±0.23	58.30	34.00	4.34±0.33	67.81
35.00	6.53±0.25	60.19	35.00	4.39±0.25	68.59

Table IV-1. One-way ANOVA of the net photosynthetic rate at the optimum temperature for photosynthesis of three grass species.

SOURCE	DF	SS	MS	F
Among groups	9	634.554	70.506	84.82**
Error	70	58.186	0.831	
Total	79	692.740		

** = $P \leq .01$

Fig. IV-7. Net photosynthesis (mg CO_2/g DW/h) and corresponding optimum temperatures for photosynthesis of three grass species grown at two temperature regimes. Values are the mean of ten replicates. Differences in intensity and pattern in tone used for net photosynthesis values indicate significant differences between rates.


Grown at 10°/5°
Grown at 25°/5°
20
16
Optimum Temp.
12
°C
8
4
2
4
6
8
10
12
mg CO_2/g DW / h
2700 m
2300 m
2000m
Poa alpina
Poa pratensis
Trisetum spicatum

(3) When plants of *P. alpina* were grown in the warm regime, the optimum temperature for photosynthesis did increase, but photosynthesis decreased significantly (except for the population from 2000 metres, in which the optimum remained stable and photosynthesis decreased). This same trend was also found in *P. pratensis*.
(4) When plants of *T. spicatum* were grown under the warm regime, the optimum photosynthetic temperature increased, and the optimum rate of net photosynthesis remained stable. (5) There was no significant difference between the three populations of *Poa alpina* with respect to the amount of carbon dioxide fixed, or to the optimum temperature of photosynthesis.

Often the shift in the temperature optimum dependent on growth temperature is used as an index of acclimation potential. While this is certainly one measure of acclimation, a more ecological assessment should consider photosynthesis rates, or the ability to maintain a similar carbon balance under dissimilar growth temperatures (Mooney 1980, Billings *et al.* 1971). The index Mooney suggests is the ratio of the photosynthesis rate at one growth temperature to the rate at another growth temperature. A value of one indicates perfect acclimation. These values were calculated for the three species and these data are given in Table IV-4. None of the species had perfect acclimation. *T. spicatum* had the lowest index, and thus the best degree of acclimation; *P. alpina* had the highest ratios, especially the populations from 2700 and 2000 metres, and thus the poorest acclimation.

The data on reduction of net photosynthetic rates after exposure to sub-zero temperatures given in Table IV-5 shows that 24 hours after exposure, the majority of grasses still have at least a 50% reduction in photosynthetic rate. A one-way ANOVA done on this data (Table IV-6) indicates that there is a significant difference among the species. From Table IV-5 it can be seen that *P. alpina* from 2000 metres, when grown under the warm regime has less percentage inhibition that when grown under the cool regime and less inhibition than *P. alpina* from 2300 meters or *P. pratensis* grown under

Table IV-4. Mean net photosynthetic rates at normal CO_2 and full light of whole plants of three grass species grown at two different thermal regimes.

Species	Growth Temperature (°C)	Analysis Temperature (°C)	Net Photosynthetic Rate ($mgCO_2$ $g^{-1}DW$ h^{-1})	Ratio of rates of cold vs. warm acclimated plants (cold/warm)
oa alpina 2700 m	10/5	10	5.42	2.72
	25/5	25	1.99	
oa alpina 2300 m	10/5	10	5.96	2.14
	25/5	25	2.78	
oa alpina 2000 m	10/5	10	4.96	2.54
	25/5	25	1.95	
oa pratensis	10/5	10	9.69	1.14
	25/5	25	8.51	
risetum spicatum	10/5	10	6.43	1.10
	25/5	25	5.62	

Table IV-5. Reduction in net photosynthesis, expressed as % of maximum rate (mg CO_2/g DW/h), 24 h after exposure to sub-zero temperatures. All values are the average of 10 replicates.

Species	Growth Temperature	Measurement Temperature	% of maximum rate before cold-exposure	% of maximum rate after cold-exposure	% reduction
P. alpina (2700m)	10°C/5°C	8.50	92.98	32.19	26.08
		15.75	90.11	64.03	26.44
		19.25	79.86	53.42	60.79
	25°C/5°C	——[a]	——	——	——
P. alpina (2300m)	10°C/5°C	7.00	87.66	16.94	70.72
		12.00	100.00	27.96	72.04
		16.00	92.00	32.07	59.93
	25°C/5°C	6.00	90.83	67.05	23.78
		9.00	97.99	87.39	10.60
P. alpina (2000m)	10°C/5°C	4.00	79.00	40.95	38.05
		13.00	96.22	51.09	45.13
		20.25	86.88	38.37	48.51
		23.50	97.61	44.14	53.47
	25°C/5°C	7.50	98.50	12.26	86.24
		14.25	100.00	10.95	89.05
		27.50	55.04	-0.17[b]	55.04

a. Data missing
b. Plants were respiring. See text.

Table IV-5. (Continued)

Species	Growth Temperature	Measurement Temperature	% of maximum rate before cold-exposure	% of maximum rate after cold-exposure	% reduction
Poa pratensis	10°C/5°C	5.50	81.31	21.67	59.64
		7.75	84.21	35.49	48.72
		30.00	80.20	37.71	42.49
	25°C/5°C	8.75	78.54	41.42	37.12
		15.25	90.30	47.48	42.82
		24.00	94.72	38.06	56.66
Trisetum spicatum	10°C/5°C	13.75	99.53	56.45	43.08
	25°C/5°C	10.00	90.00	50.23	49.86
		21.00	96.41	56.41	40.00
		31.00	76.72	57.19	19.53

Table IV-6. One-way ANOVA of % reduction in net photosynthesis 24 hours after exposure to subzero temperatures.

SOURCE	DF	SS	MS	F
Among groups	7	6285	898	5.44**
Error	16	2640	165	
Total	23	8925		

** = $p \leq 0.01$

the cool regime. Further experiments would be necessary to further clarify the differences.

A one-way ANOVA of the dark respiration rates at 20° C indicated that there was a significant difference among the three species (Table IV-7). A comparison of dark respiration rates at 20°C of the three species grown under the two growth regimes is given in Figure IV-8, based on Tables IV-8 and IV-9. The *P. alpina* population from 2700 metres grown in the cold regime, has a significantly higher respiration rate that any other population or species. There is no significant difference between the other *P. alpina* populations and any other species; nor is there a significant difference between the rates of *T. spicatum* and *P. pratensis*. In *P. pratensis* the rate of dark respiration increased as growth temperature increased, whereas in the native species the rate decreased as the growth temperature increased.

4.4 Discussion

4.4.1 Temperature relations of CO_2 gas exchange

4.4.1.1 Temperature relations of photosynthesis

There is a distinct difference between the temperature response curves of carbon dioxide gas exchange of the two indigenous species and that of the introduced species (Fig. IV-2 to IV-6). Both native grasses have very broad curves, indicating that they are photosynthetically active at both low and high temperatures. If the net photosynthetic rates of the three grasses at 0°C are compared when grown in the cool regime, the rates of *P. alpina* and *T. spicatum* were twice that of *P. pratensis*; even at -5°C, *P. alpina* had a CO_2 uptake rate of .4 to .8 mg/g/h, and *T. spicatum* had a rate of .5 mg/g/h, whereas *P. pratensis* was no longer photosynthesizing. Under the warm regime the net photosynthetic rates of the native species had decreased, but *P. alpina* was still more active than *P. pratensis*

Table IV-7. One-way ANOVA of the dark respiration rate at 20°C of the three grass species.

SOURCE	DF	SS	MS	F
Among groups	9	146.66	16.30	57.22**
Error	87	24.78	0.29	
Total	96	171.44		

** = $p < .01$

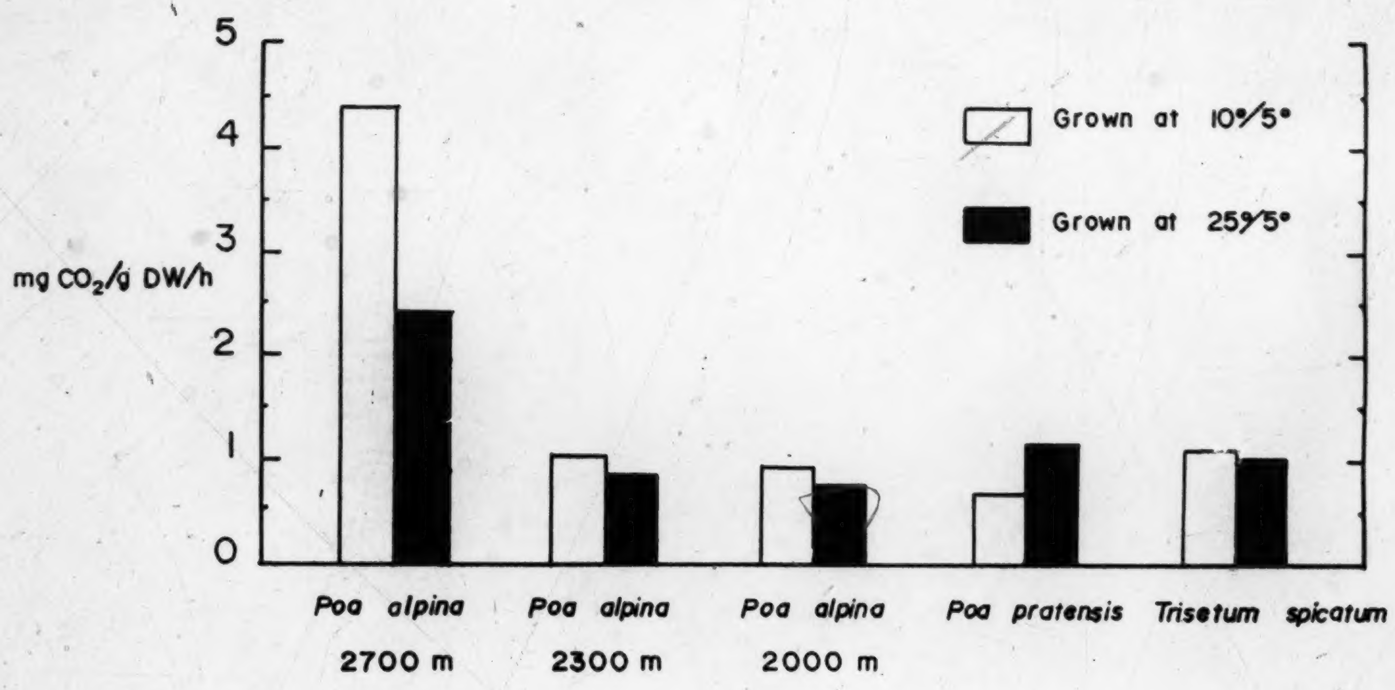


Fig. IV-8. Dark respiration rates (mg CO_2/g DW/h) at 20 C of three grass species, grown at two temperature regimes. All values are the mean of ten replicates.

Table IV-8. Respiration rate vs. temperature. Rates are expressed as absolute values (mg CO_2/g DW/h) and as % of maximum rate. Plants grown under cool regime of 10°C day/5°C night. Values are the mean of 10 replicates; standard error of the mean is given for absolute rates.

Poa alpina 2700 m			*P. alpina* 2300 m			*P. alpina* 2000 m		
Temp. °C	Absolute rate	% of max.	Temp. °C	Absolute rate	% of max.	Temp. °C	Absolute rate	% of max.
2.75	-.430±0.03	8.14	2.70	-.129±0.02	6.11			
3.50	-.469±0.03	8.88	4.15	-.221±0.01	10.47	4.60	-.414±0.06	25.71
7.50	-3.19±0.28	60.42	7.25	-.157±0.01	7.44	7.60	-.498±0.07	30.93
9.0	-3.41±0.32	64.58	9.50	-.142±0.03	6.72			
13.25	-3.59±0.20	67.99				13.50	-.669±0.28	41.55
			16.50	-.820±0.06	38.86			
16.90	-3.86±0.23	73.11				17.10	-.835±0.11	51.86
21.50	-4.47±0.26	84.66	22.70	-1.21±0.19	57.34	21.75	-.997±0.11	61.92
25.75	-4.79±0.27	90.72	29.50	-1.63±0.05	77.25	28.75	-1.32±0.13	81.99
			31.00	-2.11±0.06	100			
31.25	-5.28±0.28	100				34.25	-1.61±0.13	100

Table IV-8. (Continued)

Poa pratensis			*Trisetum spicatum*		
Temp. °C	Absolute rate	% of max.	Temp. °C	Absolute rate	% of max.
2.25	-.149±0.14	9.25			
4.75	-.150±0.02	9.32	4.50	-.335±0.06	19.71
			6.0	-.369±0.07	21.71
8.25	-.276±0.04	17.14	8.25	-.555±0.07	32.65
			10.50	-.731±0.08	43.00
11.25	-.389±0.04	24.72			
			13.0	-.815±0.06	45.94
14.50	-.498±0.04	30.19	14.25	-.773±0.07	45.55
18.50	-.725±0.07	45.03	19.0	-1.07±0.08	62.94
			22.50	-1.41±0.08	82.94
26.50	-1.21±0.14	75.16	26.50	-1.54±0.22	90.59
			28.50	-1.09±0.04	64.12
33.75	-1.61±0.16	93.17	32.5	-1.33±0.02	78.23
35.0	-1.50±0.10	100	36.75	-1.70±0.09	100

Table IV-9. Respiration rate vs. temperature. Rates are expressed as absolute values (mg CO_2/g DW/h) and as % of maximum rate. Plants grown under warm regime of 25°C day/5°C night. Values are the mean of 10 replicates; standard error of the mean is given for absolute rates.

Poa alpina 2700 m			*P. alpina* 2300 m			*P. alpina* 2000 m		
Temp. °C	Absolute rate	% of max.	Temp. °C	Absolute rate	% of max.	Temp. °C	Absolute rate	% of max.
1.90	-.973±0.15	22.63	-4.0	-.078±0.07	5.77	-1.0	.046±0.08	4.83
2.25	-1.05 ±0.17	24.42	0.5	-.260±0.06	19.26			
5.15	-1.27 ±0.10	29.53	3.5	-.060±0.08	4.44			
6.75	-1.50 ±0.21	34.88				6.5	-.356±0.19	37.39
8.75	-1.81 ±0.24	42.09	9.0	-.325±0.20	24.07			
10.75	-1.94 ±0.25	45.12						
						12.50	-.629±0.16	66.07
			15.5	-.767±0.24	56.81			
16.75	-2.02±0.21	46.98	16.5	-8.95±0.15	66.30	16.25	-.85 ±0.19	89.29
			17.0	-9.13±0.21	67.62			
			17.5	-1.34±0.25	99.26	20.00	-.952±0.30	100
23.75	-2.84±0.26	66.05						
28.25	-3.48±0.32	80.93						
33.0	-4.30±0.33	100						

Table IV-9. (Continued)

Poa pratensis			*Trisetum spicatum*		
Temp. °C	Absolute rate	% of max.	Temp. °C	Absolute rate	% of max.
5.0	-.834±0.17	40.29			
6.5	-8.85 ±0.15	42.75	6.0	-.474±0.06	36.07
			7.25	-.479±0.04	42.77
			9.50	-.604±0.04	53.94
17.0	-1.35±0.12	65.22	17.25	-.858±0.10	76.60
20.25	-1.27±0.15	61.35			
21.50	-1.29±0.16	62.32			
			22.25	-1.12±0.08	100
26.00	-1.67±0.35	80.68			
27.5	-1.71±0.12	82.61			
31.5	-2.07±0.39	100			

at both 0° and -5° C. In contrast, *P. pratensis* had a narrow temperature response curve, with a higher optimum temperature, and thus it did not do as well at lower temperatures. This species was photosynthetically active over a much narrower temperature range. However, it had higher productivity within this limited temperature range than do the native grasses. It would be informative to study the allocation of resources within these species. Different patterns of assimilate partitioning between the native and the introduced agronomic species might be expected, as the native species' carbon balance is oriented towards ensuring continuity in an environment, whereas agronomics have been manipulated by man with the goal of maximizing yield (Saugier 1983). For example, alpine plants are characterized by very large underground biomass, which is important for carbohydrate storage and soil stabilization. Preventing soil erosion is a major goal in reclamation, thus species with abundant root development would be preferable (Cuany 1974). Therefore, even though *P. pratensis* has higher photosynthetic rates, if a lower proportion of the assimilates were allocated to the root biomass than in the native species, this species would not be as successful in revegetation.

The maximum rates of photosynthesis of 5 - 6 mg CO_2 /g/h measured for these native species are comparable to those measured in several other alpine species. Mark (1975) found maximum rates in *Chionochloa* (snow tussock) species of 2.7 - 4.6 mg/g/h. The rather low optimum temperatures for photosynthesis in the native grasses (12° - 15 ° C) agree with the optima for other cool habitat species. Tieszen (1973) studied several species of arctic tundra grasses and found an optimum temperature for photosynthesis of 15°C; in these species, there is still net uptake at 0° - 5° C which is 30 - 40% of the value at 15°C. In a previous study involving *P. alpina* (Scott and Billings 1964) the optimum temperature was determined to be between 5° and 15° C, and temperatures above 20° - 25° C inhibited photosynthesis in this species. In the present experiment at about 25°C, photosynthesis in *P. alpina* populations was reduced by 20 - 50%; the reduction in *T. spicatum* was 20% under both growing regimes. In comparison, net photosynthesis in *P. pratensis* decreased only 5 - 6% at 25°C at both growth temperatures.

Although the upper temperature compensation points were not determined in these experiments, previous studies indicate that for alpine species the upper temperature limit for photosynthesis is about 35° C (Mark 1975, Pisek *et al.* 1967). The upper temperature limit would be increased in the plants grown under the warm regime. The minimum temperatures for photosynthesis in the native grasses is very near their freezing points, but above the freezing temperature for *P. pratensis* (see Table 9 in Chapter 2). In *Chionochloa* species the lower photosynthesis compensation point coincides with the leaf freezing point (Mark 1975). Photosynthesis ceases when leaves freeze and tissue is disrupted by extracellular ice formation (Bauer *et al.* 1975).

4.4.1.2 Influence of low-temperature stress on photosynthesis

When CO_2 uptake rates were measured 24 hours after exposure to sub-zero temperatures, the rates were decreased by about 50% in the majority of the grasses. The preliminary results obtained here indicate that *P. alpina* from 2000 meters is less sensitive to sub-zero temperatures than the other populations of *P. alpina* or the other two species. This result agrees with the greater degree of frost avoidance measured for this population as described above. Further experimentation is necessary to determine any differences due to species or growth regime, and a method developed that would give more control over temperature regulation at temperatures below zero. Although every effort was made to standardize the procedure used, there was variation in the temperatures attained and the duration of the treatment. For example, *P. pratensis* grown at warm temperatures was exposed to -8°C for 5.5 hours while *P. alpina* from

2300 metres grown under the warm regime was exposed to -10.0° C for four hours. This degree of variation in cold treatment may have obscured any differentiation that existed between species or growth temperature treatments. It would be important to investigate this area further, as it is essential in evaluating the decline in productivity due to stress temperatures to know how long the impairment lasts (Bauer *et al.* 1975), and this could be an important criterion in selecting the most successful reclamation species for areas with periodic frosts in the growth season. Kishitani and Tsunoda (1974) suggest that the stability of photosynthesis after cold temperature stress can be used as a characteristic for selecting breeding lines in rice plants, since differences are observable within a few days. This technique is applicable to selection of reclamation species. Field studies show that after sharp, short frosts photosynthesis is strongly reduced but recovers gradually, as long as necrosis does not occur (Bauer *et al.* 1975, Maciejewska *et al* 1983). The severity of the depression in uptake depends on the duration and severity of the frost. The decline in photosynthesis is thought to be due to a combination of factors: (1) inactivation of enzymes involved in photosynthesis; (2) frost-induced stomatal closure; and (3) the effect of extracellular freezing on chloroplast ultrastructure (Bauer *et al.* 1975).

4.4.1.3 Temperature relations of dark respiration

In the native grass species, dark respiration rates were increased when grown in the cool temperature regime; although the change in rate was significant only in the *P. alpina* population from the highest elevation, the trend is apparent in the other *P. alpina* populations and in *T. spicatum*. Increased dark respiration at cool temperatures has been observed before in alpine and other cool habitat species (Billings 1974, Gloser 1967, Moser *et al.* 1977) and is thought to be an adaptation that allows the plant to compensate for the cold and short growing season (Ollerenshaw *et al.* 1976, Billings 1974, Pearcy and Harrison 1974, Pearcy

1977, Pearcy *et al.* 1977). High respiration rates at low temperatures indicates that metabolic processes can be carried out at rates sufficient to ensure growth in cold temperatures (Mayo *et al.* 1977). In the introduced species, the respiration rate decreased in the cool temperature regime. This pattern often occurs in lowland species; and may prove to be a disadvantage for the introduced species if it is used in alpine reclamation, as it would not be able to commence growth as early in the growing season, nor have as high growth rates at cool temperatures as do the native species. The response of dark respiration rates was not as marked as the response by net photosynthetic rates; Scott (1970) and Mooney and West (1964) noted that dark respiration rates showed little pattern in relation to growing temperature.

4.4.2 Photosynthetic acclimation potentials

In recent years there have been a multitude of studies dealing with the influence of growth temperature on subsequent photosynthetic performance (Bjorkman *et al.* 1975, Pearcy *et al.* 1977, Mooney *et al.* 1978a and b, Mooney 1980). Most of these studies have found that preconditioning with high temperatures results in an upward shift in the optimum photosynthesis temperature, and that pretreatment with low temperatures causes the optimum temperature to decrease. Not only does pretreatment temperature affect optimum photosynthetic temperature, but it also appears to determine photosynthetic efficiency at subsequent temperatures. Mooney and West (1964) found that plants grown at colder temperatures are more efficient at those temperatures. Similar results have been found in numerous studies: growth at high temperatures increased the rates of CO_2 uptake at high temperatures but decreased the rates at low temperatures *vs* the responses of low temperature-grown plants (Armond *et al.* 1978, Pearcy *et al.* 1977, Mooney *et al.* 1978a and b). The responses of plants in the present experiments fit in with this pattern: growth at warm temperatures resulted in an upward shift in the temperature optimum. This type of response has generally been interpreted as photosynthetic acclimation. If judged on this basis, it would seem that all three grasses acclimated well to the changes in the growth regime, as the optimum temperature for photosynthesis increased when all three species were grown under the warm regime. However, as Mooney demonstrated (1980), in many of the previous studies as well as the present one, the shift in the temperature optimum is often accompanied by conteracting changes in photosynthesis rates, resulting in an overall decrease, not an increase in photosynthetic performance. The recent work of Pearcy (1977, Pearcy *et al.* 1977) with coastal ecotypes of *Atriplex lentiformis* provides a good example of this process. When grown under a cool regime (23°C) the rate of photosynthesis at 40°C was 60% of the maximum, which occured at 32°C. If this same clone was grown at 43°C, the optimum photosynthetic temperature increased to 40°C, but the absolute rate at 40°C was only half of the rate at 40° Cin the plant grown at 23°C. Pearcy (1977) and Mooney(1980) both conclude that these results are not evidence for photosynthetic temperature acclimation, but rather indicate that the plant is incapable of responding positively to, and is actually being injured by, the change in temperature regime. This is the situation in this experiment; as shown in Table IV-4, growing the *Poa alpina* plants under a temperature regime of 25°/5°C did cause an increase in optimum temperature for photosynthesis, but the overall photosynthesis decreased markedly. This occured in two of the three populations, indicating not temperature acclimation , but an inability to adjust the photosynthetic apparatus to the higher temperatures. The optimum temperature of photosynthesis of the *P. alpina* population from 2000 metres did not shift upwards, but the net rate of photosynthesis did decrease. *P. pratensis* also had lower rates of net photosynthesis under the warm regime, but the decrease is not as large as for *P. alpina*. Only *T. spicatum* displayed true photosynthetic acclimation: an increase in growth temperature caused an increase in optimum temperature, but the rate of photosynthesis remained stable. (Fig. IV-7). The differences in photosynthetic performance at low and high temperatures appear to be related to changes in ribulose-1,5-bisphosphate carboxylase activity in leaves (Pearcy *et al*. 1977.) Mooney *et al* (1978b) concluded that acclimation is primarily the result of changes in the intrinsic photosynthetic characteristics of a species in response to a change in environmental conditions. These authors recognize two separate effects of growth temperature regime: (1) an increased capacity for low temperature photosynthesis, probably by increasing the quantity of rate-limiting enzymes such as RuBP carboxylase; (2) an increased photosynthetic rate at high temperatures, related to an increase in the thermal stability of key components of the photosynthetic apparatus. These changes in the photosynthetic system of the plant represent a direct effect of growth temperature on CO_2 gas exchange characteristics; Ku and Hunt (1973) suggest that growth temperature can also influence CO_2 gas exchange characteristics indirectly through changes in the developmental status of the plant.

4.4.3 Ecotypic differentiation

The data presented in Fig. IV-2 to IV-6 and IV-7 indicate that there is no evidence for ecotypic differentiation among the populations of *Poa alpina* growing along an elevational gradient. Nor do dark respiration rates under both growth regimes indicate the presence of any agamotypes, with the possible exception of the population from 2700 metres, which had a dramatically higher dark respiration rate under cool regime temperatures than did the other two populations. Since there is no significant overall difference between the three populations the answer to the question, "What is the relative effect of phenotypic temperature acclimation on metabolic rates as compared to the agamotype effect?" indicates that there is no agamotype effect in *Poa alpina* from the three sites with respect to the parameters studied. Kuramoto and Bliss (1970) found that the photsynthetic response to temperature of five species did not differentiate the species-habitat relationship well. A study of several populations of Northern white cedar by Musselman *et al*. (1975) revealed very little genetic variation in photosynthesis among populations. These results agree with those of Williams and Kemp (1976) who found only limited development of ecotypes in an introduced weed species which invades disturbed sites in a wide variety of habitats. Adams *et al* (1975) demonstrated that there is a lack of evidence for ecotypic differentiation with respect to thermal adaptation of net photosynthesis among populations of *Trientalis borealis*. There are several possible reasons why there was no evidence for variation among the *Poa* populations: firstly, metabolic characteristics of locally differentiated populations are influenced more by the growth regime than are morphological characters (Billings *et al*. 1971). The optimum temperatures for CO_2 exchange by several temperate grass varieties are influenced by growth environment rather than by species or climatic origin (Charles-Edwards *et al*. 1971). In these experiments, only metabolic characters were examined, and it is possible that they adjusted to the growth regimes. Thus, if the morphological or flowering characters of the different populations had been examined, they may have reflected any agamotypic variation that was present. Secondly, any contrasting characteristics of locally adapted populations would be expected to reflect the selection pressures imposed by the respective environments (Treharne and Eagles 1970). The populations of *P. alpina* studied were taken from a simple elevational gradient on Fairview Mountain; the original habitats of the three populations thus may not have been different enough to cause directional selection resulting in the formation of agamotypes. It is also possible that the seed dispersal ranges of the three populations overlap. Further experimentation using populations from more diverse or geographically isolated areas may reveal variation between populations. Demonstration of the existence of agamotypes may therefore require an increase in sample size, or in the precision of the experimental techniques, as suggested by Adams *et al*. (1975).

4.5 Conclusions

As expected, the native grasses had lower optimum temperatures for photosynthesis than did the introduced species; they also were active over a broader temperature range than was *P. pratensis*. The native species tended to have higher dark respiration rates in cool temperatures. Together, these adaptations would give the native plants the advantage in revegetation of alpine disturbed areas where the mean temperature during the growing season is low. However, as indicated by the field data in the General Introduction, the temperatures on open disturbed sites in the alpine can sometimes be very high, and at temperatures above 20°- 25° C the native grasses had greater reduction in net photosynthesis than did the introduced grass. *P. pratensis* had higher photosynthetic rates within its narrow favorable temperature limits than did the natives. Thus, the selection of species is rather complicated: no one species is best under all conditions. The most important factor may be acclimation potential. Only *T. spicatum* displayed true photosynthetic acclimation; in both *Poa* species the photosynthetic apparatus was unable to adjust to the higher temperature regime and maintain a stable photosynthetic rate. A plant that can achieve a high net CO_2 exchange rate over a wide temperature range may be more useful under field conditions than one with high rates at only one temperature (Ku and Hunt 1973). This would especially be true in disturbed alpine areas where the temperature and other climatic conditions fluctuate greatly over short time periods. On the basis of the gas exchange characters studied in these experiments *T. spicatum* would be the most suitable of the three species studied for use in alpine revegetation.

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5. General Conclusions

All of the experiments reported here were carried out in controlled growth chambers. In such chambers, some important elements of the natural environment may be eliminated: the plants are raised under stable conditions, uniform microenvironments, with little interaction between individuals, in contrast to the complex, and constantly changing natural environment. Obviously, therefore extrapolation from such controlled environment studies to the field situation is limited. However, controlled environment experiments are an important research tool, as they give a measure of the relative performance of different species, and enable us to predict where a given species will or will not succeed. These studies are also useful to obtain information about the climatic control of physiological processes limiting productivity (Evans 1963).

The primary goal of these experiments was to identify plant characters that would enhance reclamation. The experiments reported here indicate that there is not a clearcut, obvious answer to the problem of species selection for alpine reclamation. In the majority of the experiments, the native species were more successful under alpine conditions, as might be expected. *Poa alpina*, followed by *Trisetum spicatum*, had the best germination performance based on percentage germination, rate of germination, and early seedling growth under conditions similar to those in the alpine zone at the time of germination. *T. spicatum* had slightly higher frost avoidance than did the other two species. In terms of CO_2 gas exchange, both native species had higher photosynthetic activity at low temperatures and tended to have higher rates of dark respiration under cool growth temperatures rather than warm growth temperatures. However, as suggested by Johnson (1980), it seems the introduced species are better adapted than the native species to the post-disturbance conditions. High temperatures occur frequently in many coal-mined alpine areas, and *Poa pratensis* would have the advantage under such conditions due to its higher heat resistance and greater photosynthetic rates at high temperatures.

It must be remembered that the productivity is primarily determined by the resources of the environment (i.e. water, light, temperature), rather than by the plant species (Mooney and Gulmon 1983). In alpine areas, the low average temperature of the growing season has the greatest influence on plant growth. Thus the key to survival and propagation in alpine regions is a metabolic system that can capture, store, and utilize energy at low temperatures, in a short period of time. For this reason, the native species possess definite advantages over *P. pratensis*, as they have lower optimum temperatures for photosynthesis and their dark respiration rates at cool temperatures would ensure that these species can utilize energy during the cool growing season. Since the mean air temperature in the alpine zone fluctuates markedly, especially on open disturbed sites, the most successful species would be that with a stable photosynthesis performance over a wide range of temperatures. In addition, this stability may be only of limited significance if not associated with an ability to accomodate short-term temperature changes encountered in the field (Ku and Hunt 1973, 1977). *T. spicatum* was the species with the highest acclimation potential, and in fact, possessed close to perfect acclimation. *T. spicatum* was also the species most able to avoid the freezing temperatures that can occur during the growing season. Therefore, based on the factors investigated in these experiments, *T. spicatum* shows promise of being the most suitable of the three species investigated for revegetation of alpine disturbances. However, each species studied here possess unique adaptations which give that species advantages over the others under different conditions and at different stages of the life cycle. Thus reclamation would be enhanced by using a mixture of grass species whose adaptations could compensate and complement each other, rather than relying on any one species.

There was little evidence for formation of locally adapted populations of *P. alpina*. Further studies dealing with different physiological or morphological traits may reveal some agamotypic differentiation, but from the results of these experiments, populations of *P. alpina* are not so intimately adapted to their local habitat that attempts to grow them in other alpine regions would be unsuccessful. The use of this native species would not appear to be complicated by the existence of local varieties.

A secondary goal of this research was to determine possible applications of the results to revegetation techniques. Laboratory measurement of CO_2 rates seems to be an efficient method of screening plant species for suitability in revegetation projects. The germination characteristics studied here also have a bearing on direct seeding strategies used in reclamation. The aim of these strategies is to get seedlings as well established as possible before the growing season ends. As indicated in these experiments, scarification of seeds increased both percentage germination and the rate of germination, and extends the optimal temperature range for germination.

Alpine areas have become so important to man for economic and aesthetic reasons that it is certain the rate of development in these areas will be maintained, if not increased. Obviously, man's development of these areas will result in damage to the alpine ecosystem. The realistic answer to this problem cannot be to discontinue use, but rather to develop the necessary skills and technology required to actively manage and maintain these regions.

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6. Appendix

6.1 Climate-Diagrams

A climate-diagram is used to graphically represent the climate of an area, showing its seasonal course. In the upper left-hand corner is the station, its elevation, and the number of years that observations have been made at that site. The numbers in the upper right-hand corner indicate the mean annual temperature (in ° C) and mean annual precipitation (in mm). The horizontal axis is months of the year, with the warmest season in the centre of the diagram. The solid black portion of this axis represents months with mean daily minima below 0° C; the diagonally shaded areas represent months with absolute minima below 0° C (*i.e.* those in which frosts occur). The open area of the axis represents the months with absolute minima above 0° C. The upper curve is mean monthly precipitation (one division=20 mm); the vertical shading represents a relatively humid season. The lower curve is mean monthly temperature (one division=10° C). The two values to the left side of the temperature axis are the mean daily temperature minimum of the coldest month and absolute minimum temperature. For further information, see *Vegetation of the Earth*, by H. Walter, 1979. Springer-Verlag, New York.

6.2 Field Temperature Measurements

The folowing temperature data was collected at Mountain Park West Mine using a Campbell Scientific CR-21 Programmable Micrologger during July and August, 1984. Three output tables were generated, which are integrated in into the one printout shown here. The Table number is given in Column 1; Table 1 contains the average temperature recorded by each temperature probe over a 15 minute interval; Table 2 contains the temperature maxima and time of the maxima recorded by each probe over a 24 hour period; Table 3 contains the temperature minima and time of the minima recorded by each probe over a 24 hour period. The weather affected the time-recorder of the Datalogger, such that all times are 12 hours out, *i.e.* 0500 h should be 1700 h. For July data the columns are as follows; for Table 1: Column 1 = Output table number; 2 = Julian Day (day 211= 29 July 1984); 3 = Time; 4 = probe located at 8 m northwest on transect; 5 = probe at 2.0 m height; 6 = probe at 3 m NW; 7 = probe at 1.0 m height; 8 = probe at 0.5 m height; 9 = probe at 8 m SE; 10 = probe at 3 m SE.

For Table 2, the columns are as follows: Column 1 = table number 2 = maximum for 8 m NW probe; 3 = time of maximum for 8 m NW probe; and so on. The columns in Table 3 are arranged the same as those in Table 2: Column 1 = table number; 2 = minimum for 8 m NW; 3 = time of minimum at 8 m NW; and so on.

The Datalogger did not operate well in the cold, damp weather conditions of August; some data points are missing, and recorded as question marks. For the August data the columns are as follows: Column 1 = Output table number; 2 = Julian day; 3 = Time; 4 = probe at 8 m NW; 5 = probe at 3 m NW; 6 = probe at 0.5 m height; 7 = probe at 1.0 m height; 8 = probe at 2.0 m height; 9 = probe at 3 m SE; 10 = probe at 8 m SE. Tables 2 and 3 are arranged as described above for July data collection.

July 1984

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7 25.36  16 1242.
1 0001.  02 0208.  03 1244.  04 0.059  05 25.56  06 25.44  07 001 0001.  02 0211.  03 0515.
9 21.02  10 23.36
1 0001.  02 0211.  03 0530.  04 18.66  05 16.23  06 16.53  07 15.76  08 15.87
9 18.51  10 21.77
1 0001.  02 0211.  03 0545.  04 17.02  05 15.38  06 16.42  07 15.66  08 15.67
9 17.27  10 19.95
1 0001.  02 0211.  03 0600.  04 15.13  05 13.82  06 14.76  07 14.54  08 14.06
9 15.04  10 16.77
1 0001.  02 0211.  03 0615.  04 13.66  05 13.76  06 13.47  07 13.86  08 13.23
9 13.84  10 16.11
1 0001.  02 0211.  03 0630.  04 14.18  05 14.39  06 14.04  07 14.43  08 14.39
9 14.53  10 17.05
1 0001.  02 0211.  03 0645.  04 14.60  05 14.67  06 14.74  07 14.82  08 15.04
9 14.78  10 17.66
1 0001.  02 0211.  03 0700.  04 15.63  05 14.65  06 15.37  07 15.10  08 15.20
9 14.70  10 16.60
1 0001.  02 0211.  03 0715.  04 15.63  05 15.00  06 15.43  07 15.20  08 15.28
9 15.11  10 17.20
1 0001.  02 0211.  03 0730.  04 15.95  05 14.57  06 15.53  07 15.20  08 15.27
9 15.76  10 17.07
1 0001.  02 0211.  03 0745.  04 15.83  05 14.66  06 15.61  07 14.97  08 14.99
9 15.66  10 15.61
1 0001.  02 0211.  03 0800.  04 15.09  05 14.87  06 15.76  07 15.13  08 15.22
9 16.35  10 17.21
1 0001.  02 0211.  03 0815.  04 15.74  05 14.53  06 15.48  07 14.83  08 15.11
9 15.03  10 17.66
1 0001.  02 0211.  03 0830.  04 15.26  05 15.30  06 15.75  07 14.92  08 15.73
9 14.94  10 16.37
1 0001.  02 0211.  03 0845.  04 14.60  05 14.14  06 14.40  07 14.09  08 14.31


9 13.42  10 16.84
1 0001.  02 0211.  03 0900   04 14.14  05 13.48  06 13.90  07 13.28  08 13.73
9 12.90  10 16.14
1 0001.  02 0211.  03 0915.  04 12.66  05 12.21  06 12.60  07 12.32  08 12.61
9 12.21  10 15.04
1 0001.  02 0211.  03 0930.  04 12.05  05 11.95  06 11.92  07 11.61  08 11.91
9 11.57  10 14.10
1 0001.  02 0211.  03 0945.  04 11.62  05 11.59  06 11.72  07 11.53  08 11.61
9 11.23  10 13.56
1 0001.  02 0211.  03 1000.  04 11.31  05 11.22  06 11.32  07 11.20  08 11.44
9 10.89  10 13 29
1 0001.  02 0211.  03 1015.  04 09.86  05 09.81  06 10.03  07 09.95  08 10.22
9 09.97  10 13.17
1 0001.  02 0211.  03 1030.  04 09.71  05 09.56  06 09.79  07 09.62  08 09.95
9 09.43  10 12.39
1 0001.  02 0211.  03 1045.  04 08.63  05 08.96  06 09.00  07 08.85  08 09.17
9 08.73  10 11.63
1 0001.  02 0211.  03 1100.  04 09.15  05 09.05  06 09.12  07 08 98  08 09.27
9 08.56  10 11.16
1 0001.  02 0211.  03 1115.  04 08.22  05 08.26  06 08.36  07 08.25  08 08.63
9 08.01  10 11.03
1 0001.  02 0211.  03 1130.  04 07.81  05 07.70  06 07.86  07 07.64  08 08.06
9 07.44  10 10.48
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9 6.892  10 10.30
1 0001.  02 0211.  03 1200.  04 6.972  05 07.18  06 07.16  07 07.00  08 07.41
9 6.789  10 09.86
1 0001.  02 0211.  03 1215.  04 6.060  05 6.278  06 6.266  07 6.260  08 6.530
9 6.044  10 09.86
1 0001.  02 0211.  03 1230.  04 07.21  05 07.30  06 07.39  07 07.09  08 07.82
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9 5.160  10 08.48
1 0001.  02 0211.  03 1300.  04 5.133  05 6.073  06 5.287  07 5.052  08 5.570
9 5.075  10 08.34
1 0001.  02 0211.  03 1315.  04 5.595  05 5.663  06 5.615  07 5.546  08 6.003
9 5.468  10 08.64
1 0001.  02 0211.  03 1330.  04 5.316  05 6.208  06 5.377  07 5.226  08 5.595
9 5.746  10 08.55
1 0001.  02 0211.  03 1345.  04 5.464  05 5.714  06 5.720  07 5.554  08 5.550
9 5.357  10 08.48
1 0001.  02 0211.  03 1400.  04 5.054  05 5.455  06 5.288  07 5.204  08 5.488
9 4.912  10 06.27
1 0001.  02 0211.,  03 1415.  04 5.352  05 5.385  06 5.555  07 5.282  08 5.800
9 5.172  10 08.12
1 0001.  02 0211.  03 1430.  04 4.865  05 4.889  06 5.150  07 4.950  08 5.427
9 4.806  10 08.21
1 0001.  02 0211.  03 1445.  04 4.726  05 4.611  06 4.945  07 4.723  08 5.150
9 4.602  10 07.94
1 0001.  02 0211.  03 1500.  04 4.256  05 4.700  06 4.485  07 4.415  08 4.710
9 4.282  10 07.63
1 0001.  02 0211.  03 1515.  04 4.520  05 4.782  06 4.832  07 4.651  08 5.152
9 4.504  10 07.55
1 0001.  02 0211.  03 1530.  04 4.442  05 4.671  06 4.617  07 4.545  08 4.904
9 4.248  10 07.50
1 0001.  02 0211.  03 1545.  04 4.800  05 5.036  06 4.815  07 4.847  08 5.145
9 4.302  10 07.46
1 0001.  02 0211.  03 1600.  04 4.148  05 4.486  06 4.377  07 4.348  08 4.715
9 4.034  10 07.38
1 0001.  02 0211.  03 1615.  04 4.377  05 4.571  06 4.573  07 4.529  08 5.020


9 4.181  10 07.28
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9 3.795  10 07.10
1 0001.  02 0211.  03 1645.  04 3.569  05 3 822  06 3.972  07 3.828  08 4.153
9 3.656  10 6.978
1 0001.  02 0211.  03 1700.  04 4.592  05 5.129  06 4.700  07 4.793  08 5.208
9 4.313  10 6.820
1 0001.  02 0211.  03 1715.  04 3.479  05 4.055  06 3.713  07 3.789  08 3.901
9 3.556  10 6.622
1 0001.  02 0211.  03 1730.  04 3.558  05 3.774  06 3.764  07 3.697  08 4.136
9 3.520  10 6.546
1 0001.  02 0211.  03 1745.  04 3.703  05 3.660  06 3.685  07 3.818  08 4.300
9 3.559  10 6.544
1 0001.  02 0211..  03 1800.  04 4.181  05 4.210  06 4.246  07 4.122  08 4.731
9 3.800  10 6.450
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9 3.406  10 6.367
1 0001.  02 0211.  03 1830.  04 3.216  05 3.668  06 3.597  07 3.641  08 3.741
9 3.391  10 6.316
1 0001.  02 0211.  03 1845.  04 4.001  05 4.243  06 4.166  07 4.269  08 4.485
9 3.858  10 6.488
1 0001.  02 0211.  03 1900.  04 3.896  05 4.326  06 4.215  07 4.287  08 4.401
9 4.107  10 6.656
1 0001.  02 0211.  03 1915.  04 5.423  05 5,200  06 5.882  07 6.143  08 6.560
9 5.075  10 07.16
1 0001.  02 0211.  03 1930.  04 6.487  05 5.021  06 07.89  07 08.30  08 5.702
9 5.644  10 07.72
1 0001.  02 0211.  03 1945.  04 07.57  05 07.30  06 08.65  07 10.96  08 6.868
9 07.76  10 06.85
1 0001.  02 0211.  03 2000.  04 08.57  05 08.53  06 11.63  07 11.89  08 06.26
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9 11.09   10 10.67
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9 13.84   10 12.96
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9 16.20   10 15.25
1 0001.   02 0211.   03 2100.   04 16.52   05 16.53   06 17.69   07 17.34   08 13.52
9 16.17   10 17.29
1 0001.   02 0211.   03 2115.   04 18.13   05 17.52   06 18.70   07 17.31   08 16.73
9 19.68   10 19.13
1 0001.   02 0211.   03 2130.   04 19.30   05 16.85   06 19.79   07 16.57   08 16.13
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1 0001.   02 0211.   03 2145.   04 20.56   05 19.03   06 20.90   07 18.89   08 18.26
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1 0001.   02 0211.   03 2200.   04 22.00   05 19.06   06 22.29   07 19.53   08 20.12
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9 25.40   10 25.85
1 0001.   02 0211.   03 2230.   04 23.69   05 20.59   06 23.32   07 20.83   08 21.26
9 26.11   10 27.17
1 0001.   02 0211.   03 2245.   04 23.33   05 19.10   06 22.82   07 19.64   08 20.47
9 25.36   10 26.57
1 0001.   02 0211.   03 2300.   04 24.56   05 20.90   06 23.96   07 21.34   08 22.15
9 27.61   10 28.87
1 0001.   02 0211.   03 2315.   04 24.35   05 21.14   06 23.84   07 21.61   08 22.07
9 27.26   10 29.32
1 0001.   02 0211.   03 2330.   04 26.42   05 20.84   06 25.81   07 21.64   08 23.36
9 29.65   10 31.62
1 0001.   02 0211.   03 2345.   04 26.02   05 21.65   06 25.64   07 22.34   08 23.92

9 30.29   10 32.55
1 0001.   02 0211.   03 2400.   04 27.43   05 21.98   06 26.37   07 22.82   08 23.59
9 30.78   10 33.86
1 0002.   02 26.55   03 2346.   04 23.97   05 2356.   06 28.21   07 2400.   08 24.17
9 2356.   10 24.89   11 2359.   12 31.83   13 2346.   14 34.43   15 2348.
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9 1803.   10 3.016   11 1803.   12 3.209   13 1804.   14 6.075   15 1824.
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9 31.73   10 33.76
1 0001.   02 0212.   03 0030.   04 28.83   05 22.73   06 28.26   07 24.04   08 25.19
9 33.88   10 36.86
1 0001.   02 0212.   03 0045.   04 31.06   05 22.20   06 29.16   07 23.26   08 24.84
9 33.88   10 37.19
1 0001.   02 0212.   03 0100.   04 28.46   05 23.21   06 27.42   07 24.42   08 24.62
9 32.44   10 35.22
1 0001    02 0212.   03 0115.   04 31.10   05 23.55   06 29.82   07 24.92   08 26.11
9 35.11   10 39.07
1 0001.   02 0212.   03 0130.   04 31.99   05 23.22   06 31.09   07 25.15   08 26.00
9 37.21   10 41.44
1 0001.   02 0212.   03 0145.   04 31.67   05 23.26   06 30.13   07 24.74   08 25.80
9 35.99   10 40.13
1 0001.   02 0212.   03 0200.   04 29.35   05 22.36   06 27.91   07 23.31   08 24.89
9 34.23   10 37.84
1 0001.   02 0212.   03 0215.   04 27.45   05 21.32   06 26.22   07 22.02   08 23.69
9 31.30   10 34.55
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9 24.16   10 27.27
1 0001.   02 0212.   03 0245.   04 16.26   05 16.74   06 19.06   07 18.94   08 17.67
9 20.40   10 24.30
1 0001.   02 0212.   03 0300.   04 19.56   05 19.50   06 19.83   07 20.05   08 18.42
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9 21.57   10 21.66
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9 20.29   10 21.40
1 0001.   02 0212.   03 0400.   04 21.81   05 21.17   06 21.79   07 21.61   08 20.66
9 22.03   10 23.20
1 0001.   02 0212.   03 0415.   04 23.76   05 21.34   06 23.26   07 21.48   08 22.00
9 24.63   10 26.11
1 0001.   02 0212.   03 0430.   04 20.64   05 19.86   06 20.38   07 20.22   08 20.47
9 20.79   10 23.36
1 0001.   02 0212.   03 0445.   04 20.20   05 20.01   06 20.14   07 20.08   08 20.15
9 19.88   10 21.90
1 0001.   02 0212.   03 0500.   04 20.80   05 20.55   06 21.11   07 20.89   08 20.59
9 21.74   10 22.72
1 0001.   02 0212.   03 0515.   04 22.33   05 21.03   06 22.15   07 21.30   08 21.80
9 23.03   10 24.48
1 0001.   02 0212.   03 0530.   04 25.49   05 23.10   06 24.63   07 23.23   08 23.08
9 27.04   10 28.79
1 0001.   02 0212.   03 0545.   04 22.72   05 20.68   06 22.39   07 21.30   08 21.58
9 23.27   10 25.15
1 0001.   02 0212.   03 0600.   04 14.81   05 15.27   06 14.80   07 15.89   08 15.18
9 15.86   10 18.52
1 0001.   02 0212.   03 0615.   04 14.27   05 14.53   06 14.34   07 14.72   08 14.36
9 15.13   10 17.28
1 0001    02 0212.   03 0630.   04 13.81   05 14.61   06 13.26   07 14.88   08 14.11
9 13.65   10 16.99
1 0001.   02 0212.   03 0645.   04 13.66   05 14.08   06 13.23   07 14.22   08 14.12
9 13.49   10 14.48
1 0001.   02 0212.   03 0700.   04 13.63   05 13.66   06 13.53   07 14.06   08 14.02
9 13.43   10 14.52
1 0001.   02 0212.   03 0715.   04 13.69   05 14.14   06 13.84   07 14.28   08 14.16
9 13.42   10 14.52
1 0001.   02 0212.   03 0730.   04 13.85   05 13.86   06 13.83   07 13.86   08 13.87
9 13.43   10 14.70
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